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Reading the mind in the eye: How can we use pupillometry for the assessment of neurocognitive functioning in psychiatry

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Abbreviations

ACC	Anterior cingulate cortex
ACh	Acetylcholine
BeCOME	Biological Classification of Mental Disorders
BOLD	Blood oxygen-level dependent
CG	Ciliary ganglion
DALY	Disability adjusted life years
EW	Edinger-Westphal nucleus
fMRI	Functional magnetic resonance imaging
FPN	Frontoparietal network
GWAS	Genome-wide association studies
IML	Intermediolateral cell column
LC	Locus coeruleus
MRI	Magnetic resonance imaging
NA	Noradrenaline
NIMH	National Institute of Mental Health
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
PVN	Paraventricular nucleus
RDoC	Research Domain Criteria
SC	Superior colliculus
SCG	Superior cervical ganglion
SNP	Single nucleotide polymorphism
WHO	World Health Organization

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Introductory Summary

1.1 Global burden of psychiatric disorders and its challenges within the medical field

The latest data from the World Health Organization (WHO) in 2019 indicate that one in every 8 people in the world live with a psychiatric condition, with anxiety and depressive disorders being the most common. On top of that, in 2020, the numbers increased by 26 % and 28 %, respectively, due to the global Covid-19 pandemic (WHO, June 2022). The Global Burden of Diseases, Injuries, and Risk Factor Study has estimated the health burden of 369 different diseases and injuries using a metric called disability adjusted life years (DALYs[1]). In 2019, the number of DALYs caused by anxiety and depressive disorders was estimated to be between 1.1 and 1.8 covering all ages and in the age group 10 – 24 years the number increased significantly from 3.3. to 3.7, being among the top 10 leading causes of disability in total (Vos et al., 2020). This evidence highlights the need for precise diagnostic tools and deducible effective treatment modalities for psychiatric disorders, specifically in the anxiety and affective spectrum.

In reality, the field of psychiatry struggles with the biological validity of the current diagnostic systems (Diagnostic and Statistical Manual and International Classification of Disease) and the translation of research findings into personalized clinical applications. Diagnostic criteria show a high phenotypic heterogeneity as they are not clearly separated from each other, which results in different symptom profiles leading to the same diagnosis; in turn there is a high overlap of symptoms between distinct diagnoses (Cuthbert & Insel, 2013). The problem may lie in the way psychiatric disorders are conceptualized as they are based on verbal categorization of subjectively reported symptoms rather than relying on empirical data from genetics, neuroscience, or psychophysiology. As a consequence, no central underlying disease mechanism of psychiatric disorders has been identified, and their pathogenic pathways are still elusive. Another difficulty may lay in the complexity of psychiatric disorders, as no singular

[1] DALYs quantify the loss of the equivalent of one year of full health.

underlying cause is plausible: It is rather an interaction of manifold components in constitution, etiology, and environmental exposure (Borsboom, 2017). For example, research in molecular genetics points towards thousands of common and rare genetic variants which contribute to psychiatric disorders and even further epidemiological studies have identified multiple environmental risk factors which are associated with psychopathology (Uher & Zwicker, 2017).

So far, psychiatric diagnoses are purely phenomenological and despite many proposed candidates within genetics and neuroscience, no biological marker has been found to be specific in characterizing psychiatric disorders (Scull, 2021). In psychiatry, Genome-wide association studies (GWAS) have uncovered a significant degree of polygenic architecture underlying complex traits of psychiatric disorders, in which many single nucleotide polymorphisms (SNPs) contribute to the development of one phenotype, but with relatively small effect sizes (Wendt et al., 2020). Further, GWAS have illuminated another fundamental feature of the genetic basis of psychiatric disorders, namely pleiotropy, in which a genetic variant on the gene level or at the SNP level has effects on more than one phenotype (Polushina et al., 2021). The Cross Disorder Workgroup of the Psychiatric Genomics Consortium has analyzed five major psychiatric disorders (autism spectrum disorder, attention-deficit hyperactivity disorder, bipolar disorder, major depressive disorder and schizophrenia) and found considerable evidence of genetic overlap (Cross-Disorder Group of the Psychiatric Genomics C, 2013). Pleiotropy makes it difficult to determine specific genetic causes of a particular psychiatric disorder and to interpret the results of genetic studies. One single gene may contribute to multiple disorders or combination of symptoms, which, next to polygenicity, where one trait or phenotype is influenced by multiple genes, also impedes the search for genetic markers or risk factors. This leads to the point where we face the challenge of not only high heterogeneity and low discriminatory power within symptoms and syndromes, but also in the underlying biological factors.

The introduction of non-invasive brain imaging technologies, such as magnetic resonance imaging (MRI), measuring grey and white matter volume or neural activity through blood-oxygen-level dependent (BOLD) signal in functional MRI (fMRI) initially paved the way for the identification of neuropathological underpinnings of psychiatric disorders (Jollans & Whelan, 2018). In psychiatry, for example, fMRI studies mostly focused on characterizing neural activity within the context of constructs relevant for disease, such as reward anticipation (Knutson et al., 2000), emotional processing (Hariri et al., 2002), and working memory (Owen et al., 2005). These task-based fMRI approaches highlighted differential neural activation patterns in patients compared to healthy participants. However, deeper explanatory insights are still lacking due to inconsistent findings based on small sample sizes and high false-positive rates as well as the coarseness of the methodological approach. The investigation of group differences on a macro-scale does not necessarily provide meaningful understanding for individual differences in disease etiology, and trajectories. This, once more, demonstrates the distinctive complexity of psychiatric disorders, where causal pathways appear to originate from the intricate interactions of psychological, environmental, socio-cultural, and biological factors (Nour et al., 2022).

To date, most research has focused on discrete diagnoses which are less likely to account for their multifactorial nature. In order to overcome these challenges transdiagnostic research

is coming more into focus, which cuts across traditional diagnostic categories potentially providing novel insights into how we might understand psychiatric disorders and facilitate the identification of biologically based phenotypes ("biomarkers") (Mitelman, 2019). Incorporating biological evidence into prevention, diagnosis, prognosis and treatment could improve the quality of healthcare through enabling personalized medicine and increasing remission rates for patients. From a methodological perspective, it could be essential to move beyond association studies alone, but rather towards data-driven clustering, such as unsupervised machine learning, in order to reclassify psychiatric disorders based on empirically valid and biologically based patterns of pathogenic mechanisms (Wardenaar & de Jonge, 2013).

1.2 Neurocognitive deficits as a transdiagnostic phenomenon

The occurrence of co-and multimorbidities is a principal limitation of the current nosology system of psychiatric disorders. One example of tackling these difficulties was introduced by the National Institute of Mental Health (NIMH), namely the initiative of the Research Domain Criteria (RDoC). The overarching aim is to identify transdiagnostic factors across clinical diagnoses to further guide transdiagnostic research through understanding the nature of psychiatric disorders in relation to different levels of dysfunction in fundamental psychological and biological systems. In summary, RDoC is a dimensional approach which strives to highlight biopsychological explanations for clinical symptoms (Insel et al., 2010).

Within the RDoC approach, cognitive systems are named as one of the main domains of analysis. Cumulative evidence suggests that in the context of any psychopathology neurocognitive deficits are very common, as underperformance in neuropsychological tests across neurocognitive abilities has been well documented (East-Richard et al., 2020). Within the classical nosology framework neurocognitive dysfunctions are associated with diagnoses like anxiety (Giomi et al., 2021) and affective disorders (Ahern & Semkovska, 2017), obsessive compulsive disorder (Abramovitch et al., 2013), schizophrenia (Schaefer et al., 2013), anorexia (Stedal et al., 2021) and bulimia nervosa (Hirst et al., 2017), substance use disorders (Lees et al., 2021), and personality disorders (Garcia-Villamisar et al., 2017). This underlines the notion that neurocognitive dysfunction seems to be associated with the presence of psychopathology in general, rather than being tied to a specific diagnosis or the symptom burden of a particular disorder (David et al., 2008; Doyle et al., 2018).

The p factor model by Caspi et al. (2014) proposes a general factor of psychopathology with greater explanatory power than specific factors related to single disorders. The p factor was operationalized by reclassifying symptoms of psychiatric disorders using Confirmatory Factor Analysis in a large birth cohort within a comprehensive longitudinal study design. The results indicated the best fit for a hierarchical model with general psychopathology, labeled as p factor, directly influencing all of the diagnostic symptom factors. The subordinate factors comprised latent continuous traits: externalizing, internalizing, and thought disorders. An additional analysis of neuropsychological test data revealed an association between neurocognitive dysfunction and the p factor. This finding led to the conclusion that neurocognitive dysfunction manifests across disorders, therefore, is transdiagnostically relevant and should be incorporated within the broader framework of the general p factor (Caspi et al., 2014). Follow-up

studies replicated these findings, both affirming the robustness of the p factor in reflecting psychopathology (Snyder et al., 2017), as well as highlighting the fact that neurocognitive dysfunction may be strongly linked to the overall burden of psychopathology (Martel et al., 2017).

Analogous to the p factor, Abramovitch et al. (2021) have introduced the C factor representing cognitive dysfunction. Based on their meta-analysis they could show that underperformance across neurocognitive domains was associated with multiple psychiatric disorders, which supports the general hypothesis of its transdiagnostic nature and its relation to the p factor. Specifically, in anxiety and affective disorders neuropsychological tasks tapping into the domain of executive functioning seem to be mostly affected (Abramovitch et al., 2021).

1.3 Assessment of neurocognitive functioning and its difficulties

The NIMH has introduced the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) initiative to establish a standardized and reliable framework for the assessment of neurocognitive deficits and evaluation for potential treatments to improve neurocognitive functioning. Within this approach the most promising measures were identified for the quantification of individual abilities in the main neurocognitive domains such as processing speed, attention / vigilance, working memory, verbal and visual learning, reasoning and problem solving, and social cognition which are particularly affected in schizophrenia, but also in other psychiatric disorders (Abramovitch et al., 2021; Nuechterlein et al., 2008). Mainly, the majority of these domains tap into the concept of executive functioning, which refers to a set of top-down neurocognitive processes to plan, organize, initiate, monitor, and adapt goal-directed behavior. It essentially involves higher order mental functions which support the regulation of thoughts, actions, and emotions. This makes executive functions and its deviations fundamental for mental and physical health (Diamond, 2013). Working memory, a key component of executive functions, is involved in maintenance, manipulation and retrieval of relevant information in mind and has limited capacity (Baddeley, 1992; Baddeley, 2003). Individual variability in working memory has been demonstrated to predict performance across various other neurocognitive domains (Unsworth & Robison, 2017), such as learning (Unsworth & Engle, 2005), and fluid reasoning (Kane et al., 2004). For example, in anxiety disorders, working memory deficits have been identified as a central explanatory mechanism and have been reliably associated with self-reported symptom severity (Moran, 2016).

Neuroimaging studies have identified large-scale brain networks that can be characterized by a collection of brain regions and interconnections, which are consistently activated in response to various stimuli or during resting state. Particularly, for the detection of neurobiological underpinnings of neurocognitive and affective dysfunction in psychopathology scientists have postulated that it is advantageous to apply this perspective on brain functionality and structure. Large-scale brain networks are better aligned with the heterogenous nature of psychiatric disorders and therefore could allow a more concise understanding (Menon, 2011). Meta-analyses have shown that neural correlates of executive functioning, and particularly working memory, are located within the bilateral frontoparietal network (FPN) (Owen et al., 2005; Rottschy et al., 2012). The FPN constitutes a large-scale brain network, which is responsible for higher-

order neurocognitive processes, and operates through top-down modulation. This network encompasses mainly the dorsolateral prefrontal cortex (PFC) and posterior parietal cortex including the middle frontal gyrus, inferior parietal lobule, and cingulate gyrus, and can be viewed as a functional hub, as it engages strongly with its constituent regions (Dixon et al., 2018; Marek & Dosenbach, 2018).

Up until now, for the detection of neurocognitive abilities mainly neuropsychological tests are administered as they allow the extraction of behavioral readouts with a high level of objectivity, not only in clinical settings, such as psychiatry and neurology, but also in more general applications such as the assessment of the intelligence quotient (Insel, 2014). The common conceptualization of neurocognitive impairment is manifested in statistically significant lower test scores compared to a reference sample or statistical norm (Abramovitch et al., 2021). Research into neurocognitive deficits in psychopathology aims primarily at understanding the involvement of neurocognitive functions in the etiology and manifestation of psychiatric disorders and subsequently in identifying reliable biomarkers. The assumption behind this approach is that neuropsychological test performance reflects brain abnormalities, which fits with the biomedical model and its premise that the cause of psychiatric disorders are underlying brain pathologies (Guze, 1989).

So far, no disorder-specific neurocognitive biomarker based on neuroimaging or neuropsychological tests has been found to have sufficient specificity and predictive validity (Abramovitch et al., 2021). Neuroimaging parameters are facing a low test-retest reliability through insufficient statistical power, lack of alignment of analytical pipelines and paradigms, which are impeding the significance of measured effects. Therefore, effective clinical translation of results is missing and clinical psychiatric decision making is not informed by neuroscientific data, not only because of its costly administration (Nour et al., 2022). Another difficulty is situated in the fact that neuropsychological tests are sensitive to behavioral dysfunction but are inherently unspecific and may only detect differences when impairments are really evident. Behavioral readouts of neurocognition do not allow distinguishing whether the decline is due to brain pathology, peripheral perceptual deficits or motivational and effort related factors. As such, neuropsychological tests can assess the relative difference from normative functioning, thus providing a very useful tool, but not one that allows the direct understanding of the etiology of the deficit. This leads to a limited potential for neuropsychological behavioral readouts to serve as an endophenotypic factor or neurocognitive marker in psychopathology (Abramovitch & Schweiger, 2015; Caspi et al., 2014)

It could be of high interest to foster transdiagnostic research according to the RDoC, but also move beyond neuroimaging and behavioral neurocognitive parameters, and include easy assessable psychophysiological readouts such as pupillometry. This could potentially provide a deep understanding into the brain-behavior relationship and allow a high sensitivity towards early deviations, which in turn could foster the development of diagnostic and preventive interventions. A better understanding of human neurocognition could allow the development of objective and reliable tools for the identification of specific abnormalities and thus aid in the diagnosis and treatment prediction within psychopathology and foster insights into its underlying mechanisms (Abramovitch & Schweiger, 2015).

1.4 The pupil as a readout for neurocognition and its neurobiological underpinnings

Oculomotor studies provide an ideal neuroscience model to investigate associations between neural mechanisms and behavior, as the pupil allows probing into the brain. Its biological pathways are relatively well characterized (Eckstein et al., 2017) with its two key mediators: the brainstem nucleus locus coeruleus (LC), main noradrenergic output center (releasing noradrenaline (NA), also referred to as norepinephrine), and the superior colliculus (SC) in the brain stem with its cholinergic innervations (Joshi et al., 2016). Therefore, it provides an additional measure with a high temporal resolution over and above behavioral parameters such as accuracy and response times (Luna et al., 2008).

Since the early 1960s, researchers have been studying changes in pupil size as neurophysiological markers. In particular, Kahneman and Beatty (1966) demonstrated that the pupil dilates when individuals engage in mental arithmetic tasks involving information storage, and the magnitude of dilation is contingent on the amount of information that needs to be remembered. Hence, the authors have proposed that the observation of task-evoked changes in pupil size provide a suitable indicator for shifts in mental effort, within and between neurocognitive tasks, as well as for individual between-subject differences (Kahneman & Beatty, 1966). Over the past 50 years, numerous studies have confirmed the utility of assessing pupil fluctuations as an indicator for mental effort, showing that the pupil dilates in response to task difficulty (Robison & Unsworth, 2019). However, pupil size changes are not only linked to mental effort but to a broad range of cognitive and affective processes, such as attention, memory, cognitive load (Gilzenrat et al., 2010), decision-making (de Gee et al., 2014), reward anticipation (Schneider et al., 2018), and autonomic arousal (Samuels & Szabadi, 2008). In summary, by measuring the contractions of eye muscles we can examine neural processing and provide and easy-to-access, inexpensive, noninvasive, and indirect readout of functionally relevant circuitry (Eckstein et al., 2017).

Pupil size fluctuations result from the activity of two opposing muscles: the dilator pupillae, situated in the outer parts of the iris, enlarging the pupil, while the sphincter pupillae, located in the central parts of the iris, is contracting the pupil. These muscles are controlled, respectively, by the sympathetic and parasympathetic pathways of the autonomic nervous system (McDougal & Gamlin, 2015) (Figure 1).

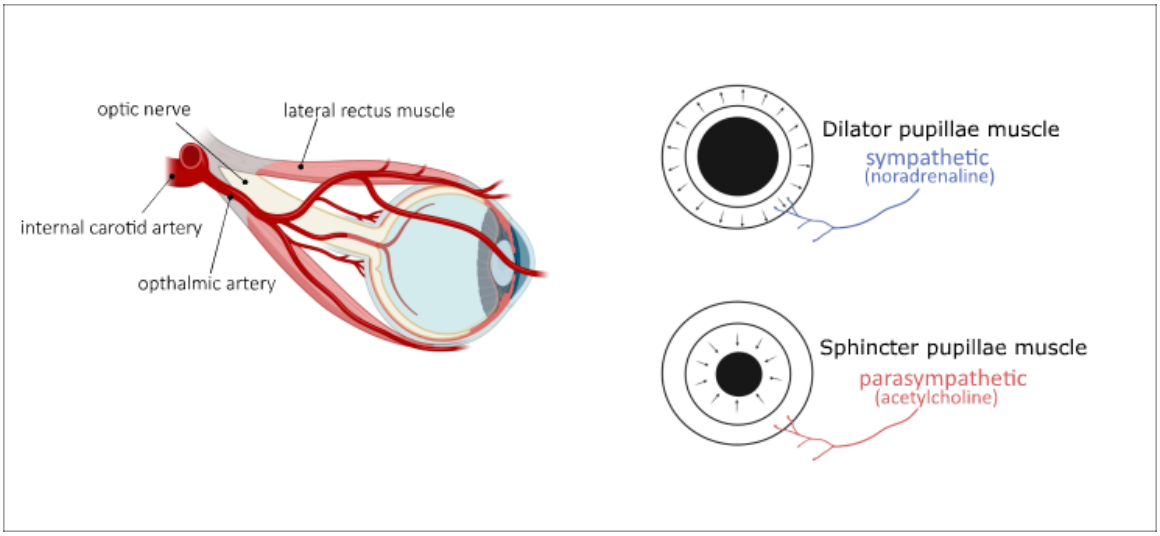

Figure 1: Axial view of the human eye and eye muscles responsible for eye movements and pupil dilation and contraction. Adapted from Eckstein et al., 2017. Figure was created with BioRender.

Within the sympathetic pathway, noradrenergic postganglionic neurons from the superior cervical ganglia (SCG) provide input to the dilator pupillae muscle, which in turn receives projections from the ciliospinal center located in the intermediolateral cell column (IML) of the spinal cord. In the parasympathetic pathway, neurons in the Edinger–Westphal nucleus (EW) in the brainstem project to the ciliary ganglion (CG). The sphincter pupillae muscle is activated by cholinergic postganglionic fibers in the CG via the short ciliary nerves. The EW is innervated by projections from the olivary pretectal nucleus, and neurons in the olivary pretectal nucleus receive direct retinal signals, which encompass inputs from intrinsically photosensitive retinal ganglion cells crucial for the pupillary light reflex (Samuels & Szabadi, 2008) (Figure 2).

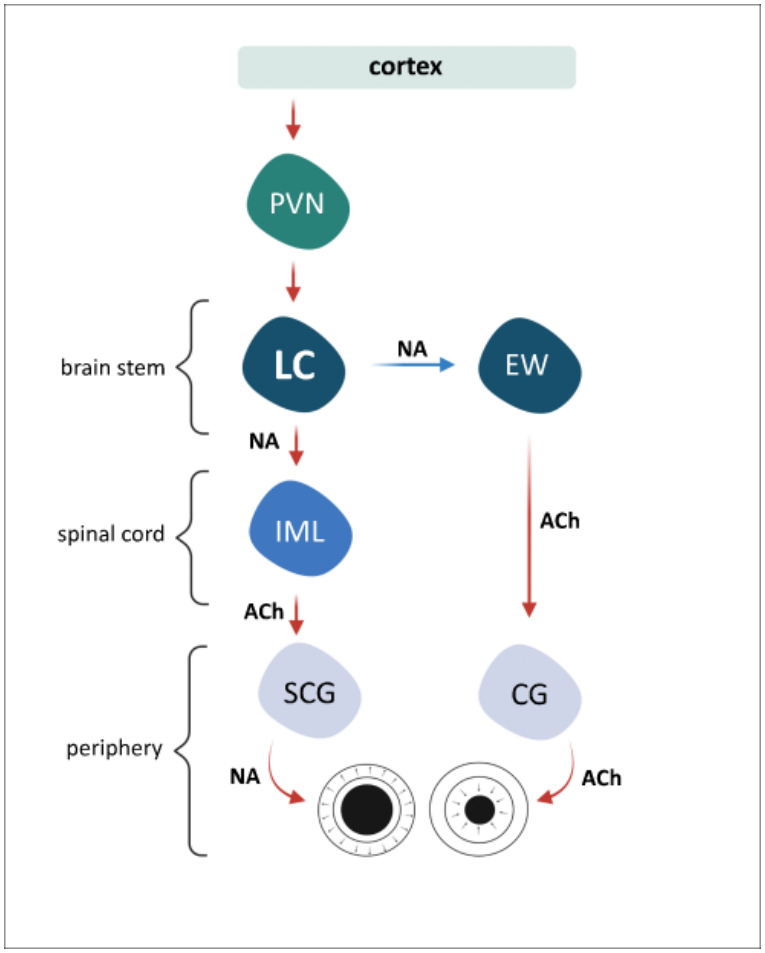

Figure 2: Regulation pathways of the human pupil. PVN = Paraventricular nucleus; LC = Locus coeruleus; EW = Edinger-Westphal nucleus; IML = Intermediolateral cell column; SCG = Superior cervical ganglion; CG = Ciliary ganglion. ACh = Acetylcholine; NA = Noradrenaline. Adapted from Szabadi (2013). Figure was created with BioRender.

Initially, researchers highlighted the role of the LC-NA system mainly in bottom-up processes, such as sensory encoding, arousal and sleep-wake cycle (Aston-Jones et al., 2007). This notion was supported by investigations, which demonstrated that LC neurons exhibited continuous firing rates during wakefulness, and reduced rates during dampened arousal, such as drowsiness and slow wave sleep (Aston-Jones & Cohen, 2005; Szabadi, 2013).

The current more prominent theory behind the LC-NA system postulates a more complex pattern involving top-down influences from cortical systems, while also modulating specific behaviors (e.g. motor responses) rather than sensory processing alone (Aston-Jones & Cohen, 2005).

Anatomical studies in rodents and non-human primates have shown that the LC has extensive projections to both cortical and subcortical regions (Szabadi, 2013). Therefore, the LC receives top-down cortical input from frontal areas such as the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) emphasizing its role in high-order processes (McBurney-Lin et al., 2019). The conclusion of these investigations emphasized that LC neurons potentially exhibit two modes of action during wakefulness, which are outlined in the adaptive gain theory (Aston-Jones & Cohen, 2005). According to this theory, the LC-NA system is responding to shifts

in task utility by modifying gain modulation of cortical processing mechanisms responsible for task performance, which, in turn, has an impact on the balance between exploitation and exploration. It synthesizes observations on particular modes of LC functioning and the influence of NA release in cortical processing. The two modes of action of the LC are: (1) the phasic mode, which is associated with rapid and transient responses to task-relevant events, and (2) the tonic mode, which refers to a sustained, baseline level of activity (Aston-Jones & Cohen, 2005).

Optimal performance in most tasks occurs at an intermediate level of arousal, and it deteriorates with either insufficient or excessive arousal. Dampened arousal results in a state of drowsiness, eventually progressing to sleep. Elevated arousal levels, triggered by the sudden occurrence of a significant environmental stimulus, can enhance performance. However, when taken to the extreme, heightened arousal may also induce distractibility and anxiety. This relationship is depicted by the Yerkes-Dodson curve which follows an inverted U-shape (Yerkes & Dodson, 1908). A similar link exists between performance and LC-NA activity. The phasic activity aids in shaping behavioral responses during task-related decision processes, while effectively filtering out responses to irrelevant events. Therefore, it boosts reactions optimizing the balance between system complexity and functional efficiency which enhances performance in the given task. Furthermore, the LC-NA system is attuned to continuous assessment of task utility, which is conveyed by input from frontal structures. As task utility diminishes, changes in tonic activity retract support for task performance, promoting alternative behaviors that explore different sources of reward. These adaptive functions are carried out through the neuromodulatory effects of NA release at cortical sites. The two modes of LC activity dynamically control the amplification of these cortical circuits, either enhancing or disengaging task-specific processes (Aston-Jones & Cohen, 2005).

Building on the neuromodulatory impact of NA release on cortical processing, the adaptive gain theory formalizes the assumption, that the phasic firing rate promotes exploitation, whereas the tonic firing rate reflects disengagement from the given task, facilitating exploration (Aston-Jones & Cohen, 2005). In phases of exploitation, task engagement is sustained while distraction is suppressed and information is processed accurately. Throughout such phases, the baseline firing rate of the LC is moderate and baseline pupil size remains stable. Phasic firing in the LC is triggered by the occurrence of salient events, causing a temporary enlargement of pupil size. Conversely, during tonic phases of exploration, task engagement is withdrawn and typically the baseline firing of the LC is elevated as well as baseline pupil size is increased. The adaptive gain theory establishes a connection between the phasic and tonic elements influencing fluctuations in pupil size and, subsequently, behavior across diverse species. (Nassar et al., 2012).

Rajkowksi and colleagues (1994) were the first in recording the firing rate of single LC neurons while concurrently monitoring pupil size changes in macaque monkeys, showing its correlative and causal evidence. Baseline pupil size in relation to its diameter during fixation and task-evoked pupil responses to acoustic sounds were positively correlated to LC activity, and the time courses of the LC firing rate and pupil size fluctuations were almost indistinguishable (Aston-Jones & Cohen, 2005; Joshi & Gold, 2020; Rajkowski et al., 1994). These results uphold the theory of a linkage between LC activity and pupil dilation. Joshi and colleagues (2016)

expanded these findings from correlative to additional causal evidence for this interconnection. Electrophysiological recordings in rhesus monkeys demonstrated that changes in pupil size, whether naturally occurring or triggered by external events, were reliably predicted at a fine temporal scale through the activation of the LC (Joshi et al., 2016).

The question arises as to which mechanisms underlie the link between the LC and pupil size fluctuations as there are no direct anatomical pathways? It has been postulated that the connection is rather based on sources of common input to the two systems (Joshi et al., 2016). Interestingly, this coupling is not unique to the LC as similar relationships are apparent in the SC and ACC and posterior cingulate cortex. However, these regions are again linked to the LC, reinforcing the general hypothesis that alterations in pupil size, not caused by luminosity factors, reflect neuronal activity mediated by the LC (Joshi et al., 2016). A more recent study by Megemont and colleagues (2022) has explored the precision with which pupil size can be used as an index for LC activity in mice. They recorded LC neurons optogenetically tagged simultaneously with changes in pupil fluctuations during a tactile detection task and successfully replicated previous findings while demonstrating a positive and monotonic correlation between pupil size and LC spiking activity. Moreover, the researchers could show that consistent optical LC stimulations produced diverse pupil responses in each trial. As a consequence, it was concluded that the variability in the coupling between the LC and pupil fluctuations is attributed to the engagement of other additional brain areas and neuromodulatory systems. (Joshi et al., 2016; Megemont et al., 2022; Reimer et al., 2016).

In humans, the quantification of LC activity with fMRI is rather challenging as the brain stem nuclei are prone to physiological noise artifacts. On top of that, the nuclei are small in relation to the spatial resolution of standard fMRI measurements, and therefore difficult to capture (Forstmann et al., 2017). Nevertheless, the first simultaneous measurement of pupillometry and fMRI in humans could reveal an association between sustained pupil size and BOLD activity in a dorsal pontine cluster, coinciding with the LC as identified through neuromelanin-sensitive structural imaging. This association was observed in both during resting state and task engagement in an oddball task (Murphy et al., 2014). In another study, where participants performed a visual divided attention task, pupillometry and functional brain activity were recorded successively. The main hypothesis was, that pupillometry as a measure of individual differences in mental effort would serve as a more accuracte predictor of LC activity than the behavioral responses alone. The results showed that changes in pupil fluctuations correlated with activity specifically in the putative LC, SC, right thalamus as well as with cortical activity in the dorsal FPN (Alnaes et al., 2014), pointing towards similar results as in non-human primates and rodents. Pupil dilation seems to be linked to functional changes in the brain that involve the LC-NA system and adjacent regions (Alnaes et al., 2014; Joshi et al., 2016). In line with this notion, the results of a subsequent study using as well individual neuromelanin-sensitive structural MRI and simultaneous pupil measurements were again largely consistent with non-human primate physiology: Phasic pupil responses were linked to activity in the brain stem, including the LC (de Gee et al., 2017). An overview of the LC projections and pupil control pathways in the human neocortex is depicted in Figure 3.

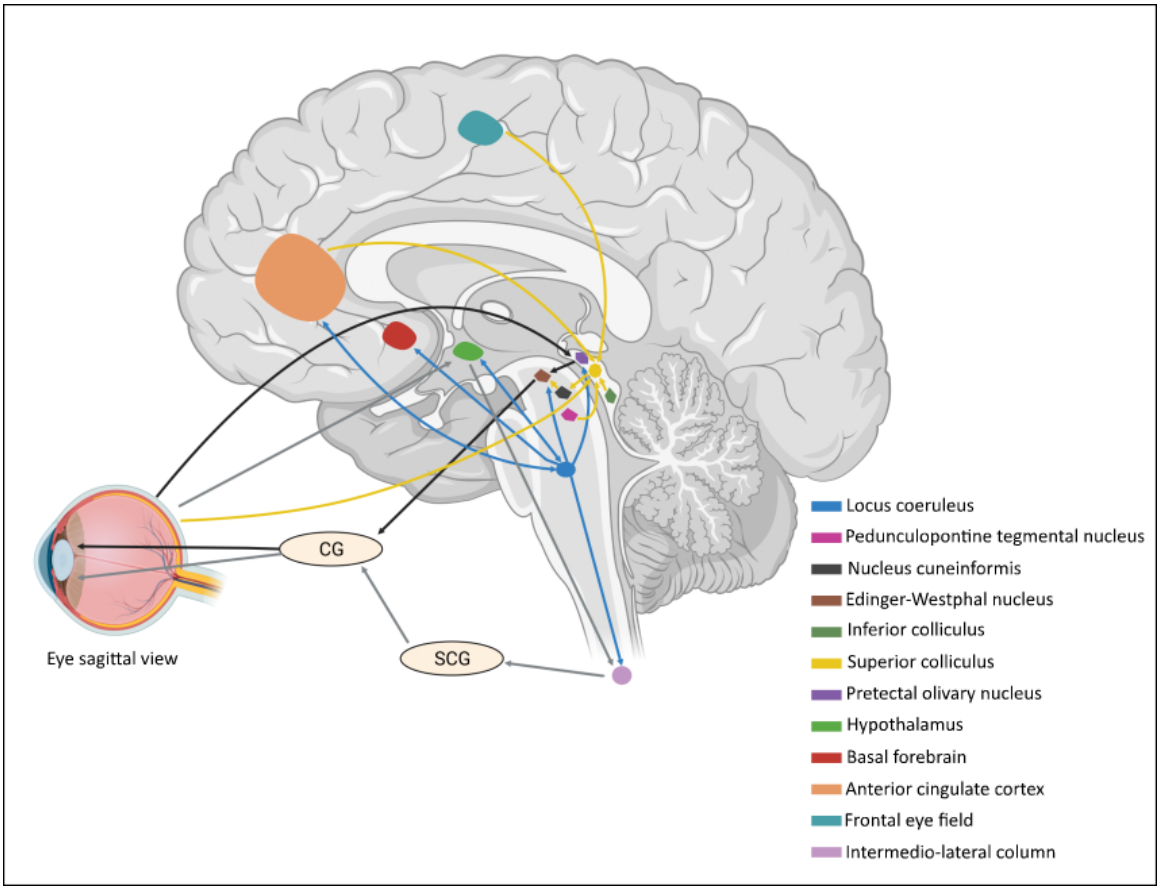

Figure 3: Brain areas and circuits involved in controlling the human pupil. Blue lines = Locus coeruleus centered pathways, yellow lines = Superior colliculus centered pathways, black lines = Parasympathetic pathways, grey = Sympathetic pathways, CG = Ciliary ganglion, SCG = Superior cervical ganglion. Adapted from Strauch et al., 2022. Figure was created with BioRender.

An updated theory on the factors affecting the pupil and its neural underpinnings in humans proposes a hierarchical model, which partitions pupil responses into five factors with low, intermediate, and higher levels. The factors on the low level are pupillary reactions to light and focal distance, the intermediate level encompasses alertness, and arousal, whereas executive functioning constitutes a high level factor. The low level factors are controlled by the pupillary muscles (Figure 1). For the arousal related intermediate factors pupil size is indirectly controlled by the LC circuit and the SC circuit, while the high level factors include circuits at all levels such as sensory and executive control areas (Strauch et al., 2022).

These findings and taxonomies highlight the potential of utilizing pupil diameter as a non-invasive readout of LC activity to obtain a clear comprehension of the involvement of the LC-NA system in brain function, particularly top-down processes, such as executive functioning. Under controlled conditions, the release of moderate levels of NA has been found to substantially influence working memory, enhancing prefrontal cortical functions through interactions with post-synaptic α-2A adrenoceptors that exhibit a high affinity for NA (Ramos & Arnsten, 2007; Sara, 2009). Moreover, an intake of NA reuptake inhibitor reboxtine as an noradrenergic enhancer improved working memory functioning highlighting its role in neurocognitive processing (Kuo et al., 2021).

Investigations on the effect of stress exposure in working memory have found that there is an evident impairment of top-down cognitive functions in the PFC when confronted with stress due to elevated catecholamine release. Simultaneously, enhancing the emotional and habitual responses of the amygdala and basal ganglia (Shansky et al., 2009). Continuous exposure to stress results in dendritic atrophy in the PFC, which correlates with impaired working memory (Hains et al., 2009). In contrast, chronic stress results in dendritic growth in the amygdala with augmentation of the NA system (Vyas et al., 2002). Elevated NA release, particularly through low-affinity α-1 adrenoceptors (and likely β-1 adrenoceptors), under stress conditions reduces the activity of PFC neurons but enhances amygdala functionality. Conversely, under non-stressful conditions, moderate NA release activates higher affinity α-2A adrenoreceptors, strengthening the PFC, weakening the amygdala, and regulating NA cell firing (Arnsten, 2000; Ramos et al., 2005). In other words, high NA engages low-affinity α-1 adrenoceptors release, and it is associated with a "stressed brain" mitigating the activity of the PFC and therefore leading to impaired working memory. Whereas, low NA release engages higher affinity α-2A adrenoreceptors which is associated with an intact neurocognitive processing and dampened amygdala activity (Arnsten et al., 2015).

Further evidence for the pupil-NA coupling was provided by using clinical depth electrodes implanted in the human amygdala while pupillometry was simultaneously recorded when participants performed a visual affective oddball task. Generally, a positive pupil-NA correlation was found and the results supported the hypothesis of a dependence between the pupil-NA coupling and various brain states as the correlation was influenced by high and low arousal levels (Bang et al., 2023).

To conclude, the LC-NA system has an impact on cortical excitability within brain regions associated with executive functioning, such as working memory. The LC is connected both anatomically and functionally to the ACC and OFC, enabling the regulation of high level pupillary responses. A more profound comprehension of these interconnections may allow the analysis of distinct neurocognitive factors by integrating psychophysiological and neural measurements (Strauch et al., 2022).

2

Aim of this thesis

The aim of this thesis was to explore the role of pupillometry during neurocognitive processing in a transdiagnostic sample including patients with stress-related disorders, mainly affective and anxiety disorders, as well as healthy control participants. The primary focus was on working memory, the core feature of executive functioning (Baddeley, 1992; Baddeley, 2003). The first study followed a methodological approach while identifying neural correlates of pupil fluctuations during a working memory task in healthy individuals. The second study built on these results, identifying computationally derived classes of pupillometric response profiles during working memory processing in psychiatric patients. The resulting clusters representing the pupillometric response profiles, were further analyzed in relation to an extensive neurocognitive assessment battery covering multiple neurocognitive domains from linguistic learning to perception and executive functioning, as well as self-reported symptoms of depression and anxiety.

3

Paper 1 | Disentangling subprocesses of working memory through simultaneous measurement of fMRI and pupillometry

3.1 Summary

The first study investigated neural correlates of pupil readouts in a working memory task in healthy control participants. This analysis was part of the Biological Classification of Mental Disorders (BeCOME) study at the Max Planck Institute of Psychiatry in Munich, Germany. The overarching goal of the BeCOME study is to identify biology-based classes of affective, anxiety, and stress-related psychiatric disorders. The results of the study should ideally facilitate the introduction of pathophysiological mechanisms of psychiatric disorders into diagnostics and then in a subsequent step improve the translational of biomedical research into individualized clinical applications within psychiatry (Brückl et al., 2020).

For this specific project, only healthy control participants were included in the analysis. They have conducted the N-back task, a reliable tool for the assessment of working memory (Lamichhane et al., 2020), while pupillometry and fMRI were recorded simultaneously. The task was designed in 8 blocks encompassing four different cognitive load conditions: fixation, 0-back, 1-back, and 2-back. Two psychophysiological readouts of the pupil diameter were extracted: (1) mean pupil size per block, and (2) the first order derivative of pupil size, namely pupil change. For the analysis of its neural correlates, mean pupil size per block and the first order derivative of pupil size within one second time bins were entered as parametric modulators to the general linear models (GLM) to analyze the baseline changes and to track fluctuations within blocks, respectively. The results showed the typical increase of pupil size with increasing working memory load. The analysis of BOLD activity associated with pupil size per block with varying cognitive load revealed a positive correlation with the FPN, the

typically observed working memory network (Rottschy et al., 2012). Pupil change (first order derivative of pupil size) on a moment-by-moment basis within one second time bins was positively coupled with activity in the salience network, i.e. the insula and ACC.

The simultaneous measurement of pupillometry and BOLD activity allowed to disentangle two subprocesses of working memory, one related to cognitive load and the other one related to the salience of the presented stimuli within the task at hand.

The combined analysis of pupillometry and BOLD activity during a working memory task with varying cognitive load sheds light on multiple levels of the hierarchical model postulated by Strauch and colleagues (Strauch et al., 2022). Pupil change on a small time scale, here within one second time intervals, is triggered by salient stimuli, which reflects arousal processes on the intermediate level. Baseline pupil size between each cognitive load condition taps into the higher-level factor indicating executive functioning and involving structures like the PFC. Overall, these findings contribute to a better understanding of working memory mechanisms and its deficits which could be of special interest for studying vulnerable groups like individuals with psychiatric disorders and neurodegenerative diseases, who tend to show such neurocognitive deficits (Huang-Pollock et al., 2017).

3.2 Contributions and reference

The study "Pupillometry tracks cognitive load and salience network activity in a working memory functional magnetic resonance imaging task" was published in Human Brain Mapping in 2022.

The project was conducted under the supervision of VS. The research project was designed by VS, PS, MC and the BeCOME study team. Data were acquired by the BeCOME study team. The data analysis was performed by JF, DP, FB. The data were interpreted by JF, VS, and PS. All authors critically revised the manuscript written by JF and VS.

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RESEARCH ARTICLE

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Pupillometry tracks cognitive load and salience network activity in a working memory functional magnetic resonance imaging task

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Abstract

The diameter of the human pupil tracks working memory processing and is associated with activity in the frontoparietal network. At the same time, recent neuroimaging research has linked human pupil fluctuations to activity in the salience network. In this combined functional magnetic resonance imaging (fMRI)/pupillometry study, we recorded the pupil size of healthy human participants while they performed a blockwise organized working memory task (N-back) inside an MRI scanner in order to monitor the pupil fluctuations associated neural activity during working memory processing. We first confirmed that mean pupil size closely followed working memory load. Combining this with fMRI data, we focused on blood oxygen level dependent (BOLD) correlates of mean pupil size modeled onto the task blocks as a parametric modulation. Interrogating this modulated task regressor, we were able to retrieve the frontoparietal network. Next, to fully exploit the within-block dynamics, we divided the blocks into 1 s time bins and filled these with corresponding pupil change values (first-order derivative of pupil size). We found that pupil change within N-back blocks was positively correlated with BOLD amplitudes in the areas of the salience network (namely bilateral insula, and anterior cingulate cortex). Taken together, fMRI with simultaneous measurement of pupil parameters constitutes a valuable tool to dissect working memory subprocesses related to both working memory load and salience of the presented stimuli.

KEYWORDS
cerebral cortex, gyrus cinguli, humans, magnetic resonance imaging, neuroimaging, pupil, short-term memory

1 | INTRODUCTION

Working memory is a core executive function (Meule, 2017) responsible for holding information in mind that is actively updated and can be recalled over a short period of time (Baddeley, 1992, 2003). It is a

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capacity limited system (Luck & Vogel, 1997), confined to the temporary maintenance and manipulation of task-relevant information, essentially contributing to higher order cognitive functioning and thus to behavior. Although working memory is typically evaluated with behavioral measures, such as reaction times (RTs) and accuracy rates, physiological measures obtained during the actual processing can provide more sensitive and biologically based readouts of individual differences within this cognitive domain (Brückl et al., 2020; Insel et al., 2010).

A substantial body of evidence has shown that pupil diameter increases with cognitive load during working memory performance (Robison & Unsworth, 2019; Unsworth & Robison, 2018; van der Wel & van Steenbergen, 2018; Zokaei, Board, Manohar, & Nobre, 2019), and experimentally high versus low working memory load can be distinguished by pupil diameter with an accuracy of up to 75% (Hogervorst, Brouwer, & van Erp, 2014). Beatty and Kahneman were the first to observe that the pupil dilates as a function of task difficulty and proposed that task-evoked changes in pupil diameter constitute a reliable physiological index of changes in "processing load" or "mental effort" (Beatty, 1982; Kahneman, Beatty, & Pollack, 1967). To summarize, pupil diameter reflects how cognitive load and attention unfold over time during cognitive processing—presumably reflecting both: task demands and individual processing differences (Alnaes et al., 2014).

In functional magnetic resonance imaging (fMRI) studies, working memory tasks were typically found to activate the frontoparietal network (FPN) across a wide range of experimental paradigms (Owen, McMillan, Laird, & Bullmore, 2005; Rottschy et al., 2012; Wager & Smith, 2003). The lateral prefrontal cortex (PFC) plays a crucial role in working memory, with the rostral-lateral PFC being related to cognitive processing during a working memory task irrespective of its specific components and the caudal-lateral PFC being related to working memory load-dependent effects (Rottschy et al., 2012). Linking physiological readouts of a working memory task with fMRI may elucidate underlying core processes. Moreover, neural correlates of pupil fluctuations in working memory have not been studied yet, particularly not in a joint fMRI/pupillometry setup.

In a single human fMRI/pupillometry study employing neuromelanin-sensitive imaging, the locus coeruleus (LC) and dorsal anterior cingulate cortex (dACC) were found to correlate with pupil diameter during rest and during performance of an oddball task (Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014). Further neuroimaging work has associated pupil dilation during resting state, fear learning, and reward anticipation with activity in the dACC and bilateral insula (the salience network) (Leuchs, Schneider, Czisch, & Spoormaker, 2017; Schneider et al., 2016; Schneider, Leuchs, Czisch, Sämann, & Spoormaker, 2018). Additionally, a more recent combined fMRI/pupillometry study showed similar results when participants undertook a steady-state attentional task, revealing a positive correlation of pupil dilation with brainstem, subcortical and cortical regions including the LC, thalamus, posterior cingulate cortex, ACC, and orbitofrontal cortex (DiNuzzo et al., 2019). This line of evidence suggests a link between spontaneous and task evoked (or modulated) pupil dilation and the salience network—sometimes also referred to as the ventral attention network, a system relevant for the detection of behaviorally relevant stimuli and the coordination of neural resources (Menon & Uddin, 2010; Peters, Dunlop, & Downar, 2016).

This implies that, while working memory is associated with FPN activity in the brain (Rottschy et al., 2012), dynamic pupil fluctuations during such processes could reflect the status of salience network involvement. Up to now, pupillometry findings in working memory tasks mainly point to pupil diameter reflecting the cognitive load (Robison & Unsworth, 2019). This notion invites the question of how these two lines of evidence can be integrated. Therefore, this study had two major objectives: first, to examine the neural correlates of varying pupil size as a function of cognitive load in a working memory task, and second, to evaluate the more dynamic pupil fluctuations within a given cognitive load condition and their neural correlates. Disentangling such subprocesses may help us to better understand working memory functioning and thus potential dysfunction in psychiatric disorders (Millan et al., 2012).

To examine this, we recorded the pupil size (equivalent to pupil diameter) of healthy participants while they performed a working memory task inside the MRI scanner, more specifically, the established N-back task that reliably activates the FPN across participants (Drobyshevsky, Baumann, & Schneider, 2006) and time (Caceres, Hall, Zelaya, Williams, & Mehta, 2009). We hypothesized that pupil size would increase in relation to increasing working memory load. Moreover, in order to investigate the blood oxygen level dependent (BOLD) correlates of working memory related pupil measures, we calculated both pupil size and pupil change (first-order derivative of pupil size) time courses throughout the block wise organized N-back task. As previous research could show a relationship between dynamic pupil fluctuations and the salience network (Leuchs et al., 2017; Schneider et al., 2016, 2018), we expected to see similar correlations during the working memory task within each block/condition. In contrast, the cognitive load dependent pupil size differences should manifest itself between the task conditions and should be closer related to the neural correlates of working memory.

2 | MATERIALS AND METHODS

2.1 | Participants

One-hundred and seven participants initially self-assigned as healthy subjects in the Biological Classification of Mental Disorders (BeCOME) study at the Max Planck Institute of Psychiatry in Munich, Germany (registered on ClinicalTrials.gov: NCT03984084) with measurements obtained up until January 14, 2020 were considered for this analysis (Brückl et al., 2020). The BeCOME study pursues the objective to identify biology-based classes of affective, anxiety, and stress related mental disorders and it also includes healthy control subjects, following the overall aim of introducing underlying pathophysiological mechanisms into diagnostics and improving translation of biomedical findings into tailored clinical applications.

Exclusion criteria for the BeCOME study in general were any current or past severe medical or neurological conditions, and the current use of psychotropic medication. Anatomical MRI sequences were inspected for incidental brain pathology, or other findings such as large arachnoid cysts that would affect the fMRI analyses. Additionally, all participants took part in the computer-based Munich Composite International Diagnostic Interview (DIAX/M-CIDI, Wittchen & Pfister, 1997), which was slightly modified for the BeCOME study through the addition of the assessment of symptoms of depression and anxiety in the past 2 weeks. For the analysis in this current study, we added a post hoc exclusion criterion: full or subthreshold (i.e., one missing symptom) current psychiatric disorder, defined as present within the past 12 months, as verified by the DIAX/M-CIDI.

Of the overall eligible sample of participants recruited as healthy subjects ($n = 107$, $M_{age} = 31.6$ years, $SD_{age} = 10.2$ years, 73 females), 38 participants were excluded due to the presence of any current full or subthreshold psychiatric disorder, 14 participants were excluded due to pupil ($n = 10$) or fMRI data ($n = 4$) not meeting the below defined quality criteria, two participants were excluded as their pupil data were not recorded due to a technical issue, and one further participant was excluded due to a general technical malfunctioning. After these exclusions, 52 healthy participants ($n = 52$, $M_{age} = 31.5$ years, $SD_{age} = 9.7$ years, 34 females) were included in our analyses. All participants in the remaining sample were non-smokers and had normal or contact lens corrected vision.

The BeCOME study protocol was in accordance with the Declaration of Helsinki and approved by a local ethics committee (Ludwig Maximilian University of Munich, reference number: 350-14). All participants provided their written informed consent after the study protocol had been fully explained and were reimbursed for their participation.

2.2 | The N-back task

In the N-back task participants view a sequence of stimuli (e.g., letters) appearing one after another and are asked to respond whenever a current stimulus (= target) matches the one from *n* steps earlier in the sequence. For the N-back task, we used a set of capital letters as stimuli (consonants B, C, D, G, P, T, W). The task as a whole contained eight blocks, each consisting of 16 stimuli, of the type 0-back, 1-back, 2-back, and fixation. We refrained from adding conditions with higher load due to the design of the BeCOME study (Brückl et al., 2020). Besides healthy participants, patients with psychiatric disorders, for example, mood disorders, were recruited for the BeCOME study in general and cognitive impairments belong to the spectrum of symptoms.

Each condition was presented once in the first half of the task and once in the second (order first half: 0-back, 2-back, 1-back, fixation; order second half: 2-back, 1-back, fixation, 0-back). In the 0-back condition, participants were instructed to react with a button press when a single prespecified target letter (i.e., W) appeared on the screen. Thus, this control condition had attentional but no working memory demand (i.e., minimal working memory load). In the 1-back and 2-back conditions, participants were asked to indicate with a button press whether a letter presented on the screen (= target) matched a letter one or two steps before, respectively. Here, the cognitive load increased with each task condition. All three aforementioned conditions encompassed four target stimuli (25%) with varying letter identities and 12 (75%) non-target stimuli per block. In the fixation condition, the capital letter X was shown repeatedly as a substitution for the letter stimuli on the screen and no action was required. This condition served as a baseline control as it included a visual input but was lacking a required motor response as well as a working memory and recognition/attentional aspect (Henson, 2007; Zhu et al., 2006).

Before each block, the respective instruction was displayed for 6 s, indicating which condition to follow. The single stimuli, as well as the capital letter X in the fixation condition, were displayed for 500 ms followed by a fixation cross displayed for 2,000 ms before the next stimulus appeared on the screen. In the first 1,000 ms of the fixation, cross display answers were collected.

All stimuli were presented using Presentation Software version 18.01 (Neurobehavioral Systems Inc., Berkeley, CA) in a central position on a monitor located about 2 m behind the end of the scanner bore, which could be seen by the participants via a mirror that was attached to the head coil.

2.3 | Experimental procedure

The N-back task was included in the fMRI session on the first BeCOME study day (Brückl et al., 2020). Before performing the task inside the scanner, participants received instructions about the N-back task in front of a computer outside the scanner by experienced technicians instructed into the BeCOME study.

To ensure that participants fully understood the N-back task, they completed a short, standardized training of the task outside the scanner room. The training phase involved each condition of the task. After assurance that the task was fully comprehended and any remaining questions were clarified, participants were positioned in the scanner.

2.4 | Behavior

To compare RTs and accuracy rates between conditions of the N-back task with varying working memory load, we computed the individual mean RTs and mean accuracy rates across respective trials and conditions. Accuracy was defined as the ratio of pressing the response button in response to targets (= hits) in time, that is, from stimulus onset until maximum 1,000 ms after end of the stimulus presentation in addition to not pressing the response button when non-targets appeared on the screen (= correct rejections) and the total number of trials. Additionally, we quantified error responses as incorrectly not pressing a button in response to targets (= missed hits) and incorrectly pressing the response button in non-target trials (= false alarms), see Results section and Figures S1–S4 in the Supplement.

For three participants, the behavioral parameters were not recorded due to technical reasons; therefore, the behavioral analyses were restricted to 49 participants.

2.5 | Pupillometry

Pupil size and gaze coordinates were recorded with an MR-compatible eye tracker (EyeLink 1000 Plus; SR Research, Osgoode, ON, Canada), which was placed at the end of the scanner bore and below the presentation monitor, such that the participant's right eye could be tracked via the head coil mirror. Pupil size data were acquired in arbitrary units with a sampling rate of 250 Hz. In order to calibrate the eye gaze position on the monitor, a standard nine-point calibration procedure was applied. Eye tracking data were processed and analyzed in MATLAB (version 2019b, MathWorks, Natick, MA). Missing data resulting from eye blinks were linearly interpolated between the last saccade before blink onset and the first saccade after blink offset. Saccade markers were provided by EyeLink software (SR Research Ltd.). After this procedure, pupil size data were smoothed by computing the mean of a 200 ms sliding window and z-transformed to control for variability in average pupil size across participants.

In order to ensure optimal data quality, datasets with more than 20% blink/eye closure-related missing pupil size values within one block of the task were excluded ($n = 8$), this rate is equivalent to more than 20% of interpolated data within one block. As strong shifts in gaze can interfere with the pupil size detection, we also checked whether the participants' gaze within one block was directed at the center of the screen. For this purpose, we determined the median of the horizontal (x) and vertical (y) gaze data over the course of the task for each participant, yielding a pair of coordinates that indicated the center of the screen on an individual level. Next, we computed the average *SD*s of the x gaze (sd_x = 105.34) and y gaze (sd_y = 91.40) shift across all participants. We defined a cut-off window by using 3.3 *SD*s around the participant's individual center coordinates. If the participant's gaze remained outside this cut-off window for more than 20% of the time within one block, the participant was excluded from further analyses ($n = 2$). The procedure of the data quality check was derived from previous literature on pupil fluctuations and their neural correlates in a number of tasks and resting state (Leuchs et al., 2017; Schneider et al., 2016, 2018, 2020). In this study, we adapted the criteria per block (instead of per stimulus) as we were interested in the between and within effects of the blocks, which were also modeled in our subsequent analysis. We also reran the main analyses with including these subjects (for results of this additional analysis see section 2.2 in the Supplement and Figures S5 and S6).

Pupil change was calculated as the first-order derivative of pupil size. This difference between two consecutive time points of pupil size, equivalent to pupil change, was calculated using MATLAB (version 2019b, MathWorks). For further pupil response quantification, we obtained the pupil maximum value in the search window of 1,000 ms (after stimulus presentation and the light reflex) to 2,500 ms (trial end). From this maximum value, we then subtracted the baseline of the respective trial defined as the mean pupil size between trial onset and 500 ms, which equals the stimulus presentation, just before the light reflex, and after the refractory period of the previous trial.

Additionally, we analyzed a potential tiring effect based on pupil size differences between the first and the second half of the N-back task, see Results section and Figure S7 in the Supplement.

2.6 | Statistical analyses of behavioral and pupillometry data

We used Bayesian inferential statistics as implemented in the software package JASP 0.12.2 (https://jasp-stats.org). We performed Bayesian one-way repeated measures (rm) analysis of variances (ANOVAs) with the N-back conditions (0-back, 1-back, 2-back, and fixation) as the within subject factor. In a Bayesian repeated measures (rm) ANOVA, different models are compared based on their likelihood given the data. In our case, model comparisons included the null model, stating that there is no effect of condition, and the alternative model with the effect of condition, stating that the conditions differ. The prior probability is equally distributed over those two options (0.5) and the updated probability after observing the data (P(M|data)) provides the relevant output for these analyses. The posterior odds represent the relative plausibility of the alternative model after observing the data, and it is equal to the Bayes factor (BF_{10}) multiplied by the prior odds. The Bayes factor quantifies the change of relative plausibility given the data. A BF_{10} of around one indicates that the observed data are equally likely to occur under both models, a BF_{10} between one and three can be interpreted as anecdotal evidence for the alternative hypothesis. A BF_{10} above three but under 10 is seen as moderate evidence for the presence of an effect in favor of the alternative model, and a BF_{10} above 10 is proposed to indicate strong evidence for the presence of an effect. Whereas, for example, a $BF_{10} < 1/3$, which is mathematically equivalent to $BF_{01} > 3$, can be interpreted as moderate evidence in favor of the null model (Wagenmakers et al., 2018). For Bayesian ANOVA post hoc tests, Bayesian *t* tests were used. To control for multiple testing, the prior probabilities were adjusted following the Westfall approach (Westfall, 1997). The calculation of the prior model odds depends on the number of respective conditions and in that way each single comparison is considered. The multiplication with the unadjusted Bayes Factor for each pairwise comparison with a Cauchy (0, $r = 1/\mathrm{sqrt}(2)$) prior, results in corrected posterior odds (van den Bergh et al., 2020). For reasons of readability, we followed a hybrid approach and also report more commonly used frequentist statistics (Keysers, Gazzola, & Wagenmakers, 2020).

2.7 | fMRI data acquisition and preprocessing

All participants were scanned in a 3 Tesla MRI Scanner (Discovery MR750, GE, Milwaukee, WI) at the Max Planck Institute of Psychiatry in Munich, Germany. For the data acquisition a 32-channel head coil,

covering 40 slices (AC-PC orientation of the slices, 96 × 96 matrix, in-plane field of view 24 × 24 cm^2, 3 mm slice thickness, 0.5 mm slice gap, resulting voxel size 2.5 × 2.5 × 3.5 mm^3, echo planar imaging [EPI], TR 2.5 s, TE 30 ms, acceleration factor 2) was used. The N-back task included a total number of 176 image volumes, of which the first four volumes were discarded to avoid non-steady-state effects. Preprocessing and analysis of the fMRI data was performed with MATLAB (version 2019b, MathWorks) using SPM12 (Statistical Parametric Mapping Software, Wellcome Centre for Human Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/SPM), and FSL 6.0 (Wellcome Centre Integrative Neuroimaging, Oxford, UK, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Preprocessing of the functional images encompassed—in the order given—(a) realignment using rigid body motion correction with the first image of the task as reference with an additional FSL based rigid body motion to calculate root-mean-squared intensity differences between volumes referred to as DVARS, based on Power et al. (2014) and the resulting dummy regressor matrix was saved for later denoising (outliers defined as values larger than 75th percentile plus 1.5 times the interquartile range) (Power et al., 2014); (b) slice time correction considering the bottom-up acquisition interleaved scheme; (c) coregistration of the time series on a specific single contrast-rich T2-weighted EPI image (details in the Supplement); (d) segmentation of this specific image using the unified segmentation algorithm in SPM to separate white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF), (e) spatial normalization entering GM and WM probability maps into the iterative DARTEL algorithm (Ashburner, 2007) using IXI study templates (www.brain-development.org) in MNI space, (f) interpolation to a voxel resolution of 2 × 2 × 2 mm^3, (g) brain extraction using the FSL brain extraction tool (BET, FSL version 6.0), and (h) spatial smoothing using an isotropic Gaussian Kernel (full width at half maximum 6 × 6 × 6 mm^3). Denoising was performed including the following set of nuisance covariates in all first level general linear models (GLM): (i) Following the aCompCor strategy (Behzadi, Restom, Liau, & Liu, 2007), five components of WM and CSF (based on segmentation mentioned in Step (d)); (ii) six motion correction coefficients from Step (a) along with their temporal derivatives; and (iii) the DVARS-based binary matrix. Subjects displaying excessive head movement during scanning—potentially causing motion artifacts—were excluded from the study ($n = 4$). The threshold for exclusion was set at 2 mm translation between two consecutive volumes.

2.8 | First level analysis

Separate first level GLMs were created for modeling pupil size and pupil change. For analyzing pupil size associated neural activity *between* conditions, we entered the mean pupil size per block (40 s time bins) as a parametric modulation within one blockwise regressor. The blockwise regressor included onset times of all blocks in the four conditions (fixation, 0-back, 1-back, 2-back) presented in the N-back task.

For analyzing pupil change associated neural activity *within* conditions, we divided the same blocks into 1 s time bins, which constituted the regressor, and entered the corresponding mean pupil change values of those 1 s time bins as its parametric modulation. For this purpose, we downsampled pupil change to 1 Hz. We decided for this approach, as downsampling to the TR (Murphy et al., 2014) before convolution with the hemodynamic response function would result in reduced temporal information, we were particularly interested in.

We used separate models for analyzing neural correlates of pupil size and pupil change in order to prevent collinearity of regressors, which would have a negative effect on statistical power as well as on the parameter estimates (Mumford, Poline, & Poldrack, 2015).

To explicitly model potential effects of condition on pupil change and to avoid the uncontrolled merge with interaction effects, we created an additional first level GLM in which we partitioned the condition regressor (that so far was represented as one single regressor) into four separate regressors (one for 0-back, one for 1-back, one for 2-back, and one for fixation) with onset times of the corresponding 1 s time bins. The equivalent mean pupil change values were entered as parametric modulation.

To examine the neural correlates of trials and their pupil responses, we created another GLM with one regressor encompassing onsets of all trials across the whole task with a duration of 2.5 s. We added the equivalent peak amplitudes in each trial as its parametric modulation.

We used parametric modulators as they provide a flexible analysis approach to disentangle the between and within block/condition effects of the pupil parameters (Leuchs et al., 2017; Schneider et al., 2016, 2018; Wood, Nuerk, Sturm, & Willmes, 2008).

All GLMs were run with nuisance regressors as stated above.

2.9 | Second level analysis

The group analyses were performed using Bayesian inference as implemented in SPM12. The contrast images of the first level analyses of all participants were used for the model, and tested with Bayesian one-sample *t* tests against zero (contrasts [+1] and [−1]) for the underlying pupil size, pupil change, and pupil peak GLM. For the statistical maps a minimum effect size of Cohen's $d = 0.2$ and a minimum Bayes factor of ~1,000 was selected (logBF = 3). In cases where relevant separate clusters merged into one larger cluster, the threshold was increased ($d = 0.5$, logBF = 3). For additional analyses, we ran a one-way ANOVA in SPM12 with one factor (condition) encompassing four levels (fixation, 0-back, 1-back, 2-back; dependent cells) as well as a logical "AND" conjunction analysis to examine to what extent the neural correlates of pupil change depended on condition. All analyses were performed in MATLAB (version 2019b, MathWorks).

The tables detailing the anatomical extent of clusters were created using the automated anatomical labeling (AAL) atlas (AAL 2 toolbox; (Rolls, Joliot, & Tzourio-Mazoyer, 2015; Tzourio-Mazoyer et al., 2002). Technically, as the AAL toolbox cannot process posterior

probability maps, we used the frequentist maps at voxelwise p_{FWE} < .001 threshold for anatomical labeling.

The background image used for depiction of statistical maps (Figures 4–7, S4–S6, S10–S16, S18, and S19 in the Supplement) was generated by unified segmentation of all T1-weighted images, followed by DARTEL spatial normalization, driven by GM and WM segments (IXI templates, default settings), and application of the flow fields to the bias-corrected whole head image. Supplemental Figure S8 illustrates the spatial normalization procedure and compares the matching of the resulting normalized functional images with the template space.

3 | RESULTS

3.1 | Behavioral results

The Bayesian one-way rmANOVA yielded very strong evidence for an effect of condition on RT with P(M|data) = 1.0, $BF_{10} = 2 \times 10^{11}$, $F_{(2,96)} = 43.1$, $p < .001$ (Figure 1a). Descriptive statistics are listed in Table 1. This shows that RT depended on the working memory load level of the respective condition in the N-back task. The adjusted posterior odds show (a) strong evidence that 0-back differed from 1-back (odds of 15), (b) very strong evidence that 0-back differed from 2-back (odds of 4×10^{10}), and (c) very strong evidence that 1-back differed from 2-back (odds of 1.7×10^{3}). Results of the Bayesian post hoc tests are listed in Table S1 in the Supplement.

Regarding accuracy, the Bayesian one-way rmANOVA showed very strong evidence for an effect of condition on accuracy with P(M|data) = 1.0, $BF_{10} = 1 \times 10^{6}$, $F_{(2,96)} = 21.4$, $p < .001$ (Figure 1b). Descriptive statistics for accuracy are depicted in Table 2. The adjusted posterior odds show (a) very strong evidence that 0-back differed from 2-back (odds of 7.4×10^{3}), (b) very strong evidence that 1-back differed from 2-back (odds of 2.4×10^{2}), and (c) some evidence for no differences between 0-back and 1-back (odds of 0.3). Results of the Bayesian post hoc tests are listed in Table S2 in the Supplement.

3.2 | Pupillometry

For pupil size, the Bayesian one-way rmANOVA yielded very strong evidence for an effect of condition on pupil size P(M|data) = 1.0, $BF_{10} = 1 \times 10^{71}$, $F_{(2,102)} = 166.0$, $p < .001$, indicating that pupil size depended on working memory load (Figure 2). The adjusted posterior odds show (a) very strong evidence that 0-back differed from 1-back (odds of 1.6×10^{5}), 2-back (odds of 1×10^{20}), and fixation (odds of 9×10^{7}); (b) very strong evidence that 1-back differed from 2-back (odds of 1×10^{13}) and fixation (odds of 4×10^{16}); and (c) very strong evidence that 2-back differed from fixation (odds of 1×10^{27}). The results for the post hoc tests are shown in Table S3 in the Supplement.

To investigate event-related pupil responses, we analyzed target and non-target trials in the three active N-back task conditions

TABLE 1 Descriptive statistics for RT

				95% credible interval	
Condition	**Mean**	***SD***	***N***	**Lower**	**Upper**
0-back	0.400	0.045	49	0.386	0.413
1-back	0.432	0.087	49	0.407	0.457
2-back	0.498	0.082	49	0.474	0.521

Note: RT is presented in s.
Abbreviation: RT, reaction time.

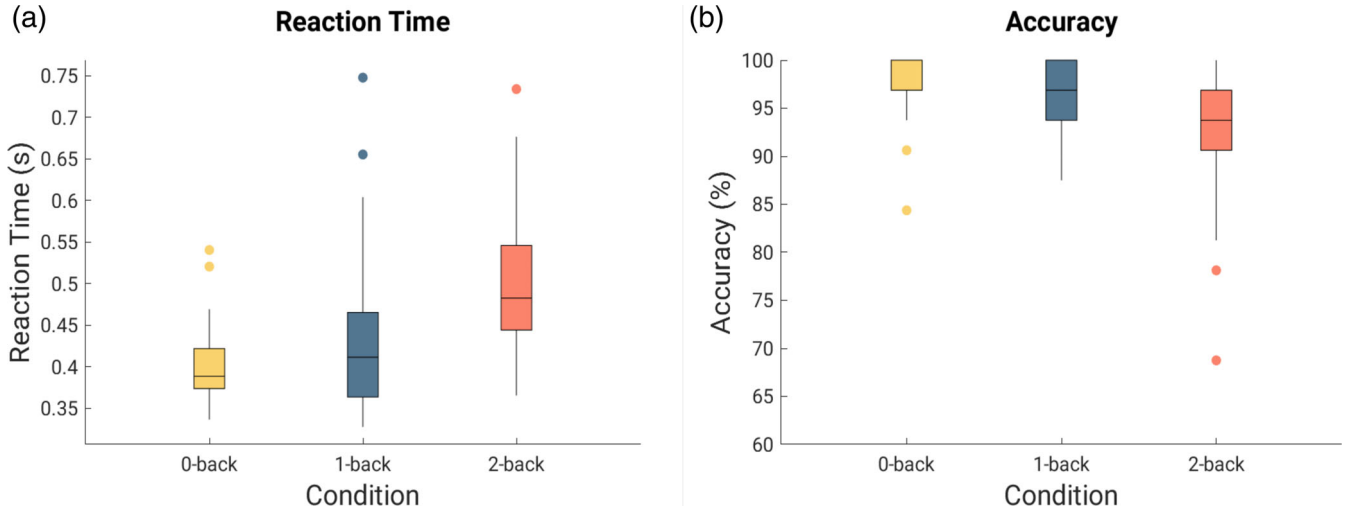

FIGURE 1 Boxplots showing (a) reaction time (RT) and (b) accuracy in the N-back task in each condition. Horizontal line within each box denotes median values; boxes extend from the 25th to 75th percentile; vertical extending lines (whisker) denote values outside the interquartile range (IQR). The upper whisker extends to the largest value no further than 1.5 × IQR and the lower whisker extends to the smallest value no further than 1.5 × IQR; dots beyond the end of the whiskers represent outliers

(0-back, 1-back, and 2-back). It becomes evident that the pupil shows a stronger dilation in relation to target trials compared to non-target trials in all three conditions (Figures 3 and S9 in the Supplement). To examine differential scores, we subtracted peak values of the non-target trials from the peak values of the target trials for each condition (0-back, 1-back, and 2-back). A Bayesian one-way rmANOVA revealed strong evidence for an effect of condition, $P(M|data) = 1.0$, $BF_{10} = 2.94 \times 10^{11}$, $F_{(1.73,88.19)} = 41.73$, $p < .001$ (Greenhouse–Geisser corrected as assumption of sphericity was violated according to Mauchly's test). The adjusted posterior odds show (a) very strong evidence that the differential peak amplitude in the 0-back condition ($M = 0.59$, $SD = 0.3$) was larger than the values for the 1-back ($M = 0.42$, $SD = 0.22$, odds of 63.79) and 2-back conditions ($M = 0.23$, $SD = 0.22$, odds of 1.89×10^8), and (b) very strong evidence that the values for the 1-back condition were larger than the vales for the 2-back condition (odds of 5.72×10^3).The descriptive statistics of pupil peaks (maximum pupil amplitude minus baseline) are provided in Table 3.

Furthermore, we analyzed the relation between pupil size and performance on a trial-by-trial basis. We first down-sampled the pupil size vector to a resolution of 10 Hz in each trial and then took the mean of the pupil size values per trial (duration of 2.5 s) of all target trials in the three active N-back conditions (0-back, 1-back, and

TABLE 2 Descriptive statistics for accuracy

				95% credible interval	
Condition	**Mean**	***SD***	***N***	**Lower**	**Upper**
0-back	0.980	0.037	49	0.969	0.990
1-back	0.970	0.034	49	0.960	0.980
2-back	0.929	0.066	49	0.910	0.948

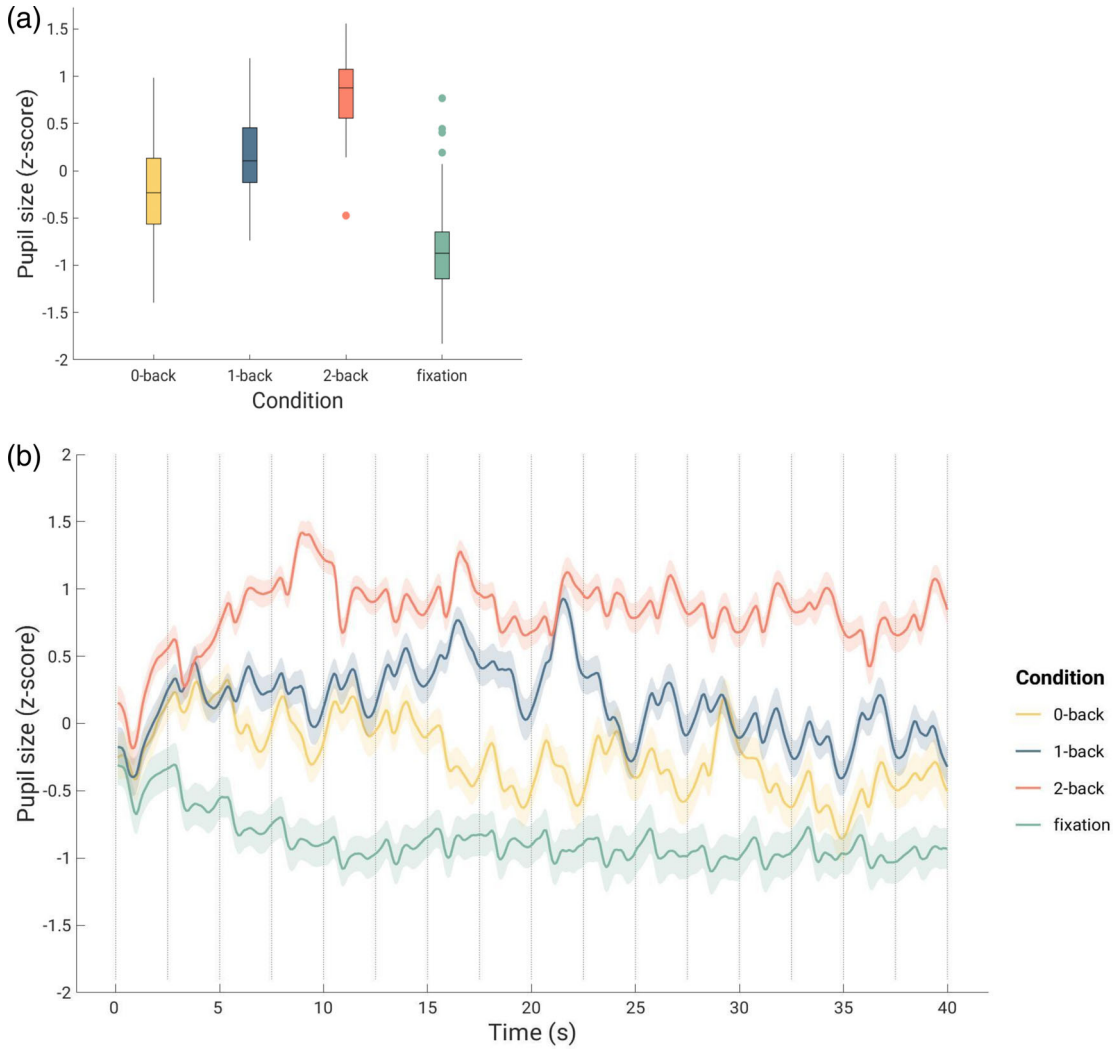

FIGURE 2 (a) Boxplot showing the distribution of pupil size calculated as the mean of pupil size values in each condition (one value per participant). (b) Mean pupil size over the time course of a task block within each condition. The x-axis represents the length (40 s) of one block. We downsampled the pupil size values to 10 Hz and calculated the mean of both halves of the task within each condition. The shaded area represents 95% confidence intervals of the mean. The gray vertical lines indicate trial onsets. The last gray vertical line at 40 s indicates the end of the block

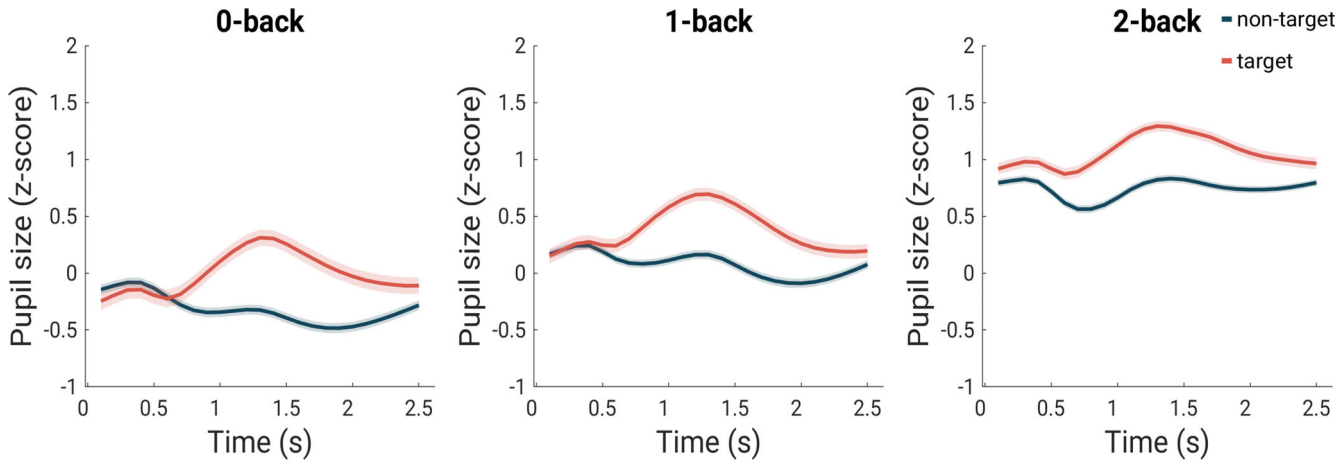

FIGURE 3 Mean pupil size in response to non-target (in blue) and target (in red) trials in the three active N-back task conditions (0-back, 1-back, and 2-back). The x-axis represents the length (2.5 s) of one trial. Between 0 and 0.5 s, the stimulus is presented, between 0.5 and 1.5 s, the response, if necessary, is collected and between 1.5 and 2.5 s is the inter trial interval. The shaded area represents 95% confidence intervals of the mean

TABLE 3 Descriptive statistics of pupil peaks

	0-back T	0-back NT	1-back T	1-back NT	2-back T	2-back NT
Mean	0.72	0.13	0.66	0.26	0.57	0.34
SD	0.36	0.13	0.33	0.21	0.33	0.22
Minimum	0.15	−0.13	0.13	−0.16	−0.04	0.04
Maximum	1.78	0.44	1.50	0.79	1.47	1.07

Note: T = target trials, NT = non-target trials, *SD* = standard deviation.

2-back) with correct responses (= hits). Then, we calculated the Pearson correlation coefficient between these trial-wise pupil size values and its respective RTs, which yielded a weak but significant (positive) correlation, $r = .23$, $p < .001$.

3.3 | Functional magnetic resonance imaging

3.3.1 | Neural activity related to pupil size between conditions

The second level GLM of the mean pupil size values per block revealed very strong evidence for positively correlated BOLD activity mainly in the FPN: the dorsolateral PFC (superior frontal gyrus, middle frontal gyrus, supplementary motor area [SMA]), ventrolateral PFC (inferior frontal gyrus), posterior parietal lobules (angular gyrus), in addition to activity in the bilateral insula, $d = 0.2$, logBF >3 (Figure 4). The reverse contrast revealed very strong evidence for negatively correlated BOLD activity in bilateral clusters of the precuneus, orbitofrontal gyrus, as well as activation in the anterior cingulate gyrus, posterior cingulate gyrus, and the lateral parietal cortex (precentral and postcentral gyrus), $d = 0.2$, logBF >3. For a detailed listing of these clusters, see Table S4 in the Supplement.

3.3.2 | Neural activity related to pupil change within conditions

The GLM with the mean pupil change values of the one-second time bins revealed very strong evidence for correlation with BOLD activity in the bilateral insula, caudate, thalamus, orbital inferior frontal gyrus, anterior and middle cingulate gyrus, as well as in the superior frontal gyrus, $d = 0.5$, logBF >3 (Figure 5). The anterior insula and the anterior cingulate gyrus are typically conceptualized as the primary components of the salience network (Menon & Uddin, 2010). The negative contrast revealed strong evidence for corresponding negative correlations with the occipital lobe, $d = 0.5$, logBF >3. For a detailed listing of these clusters, see Table S5 in the Supplement.

To examine the possibility that these correlates (Figure 5) were confounded by differences in the mean pupil change values per condition (main effect of condition on pupil change, posterior probability = 1.0), we ran two additional fMRI analyses. For the first analysis, we entered the *mean* pupil change values of each block (one value per block) into the GLM, analog to the aforementioned pupil size GLM. For the second analysis, we entered demeaned pupil change values within 1 s time bins (40 s per block = 40 values) as parametric modulation in four separate regressors (one per condition) into the model, such that there were no mean differences among the blocks anymore. For the demeaned pupil change values, the mean pupil change of each

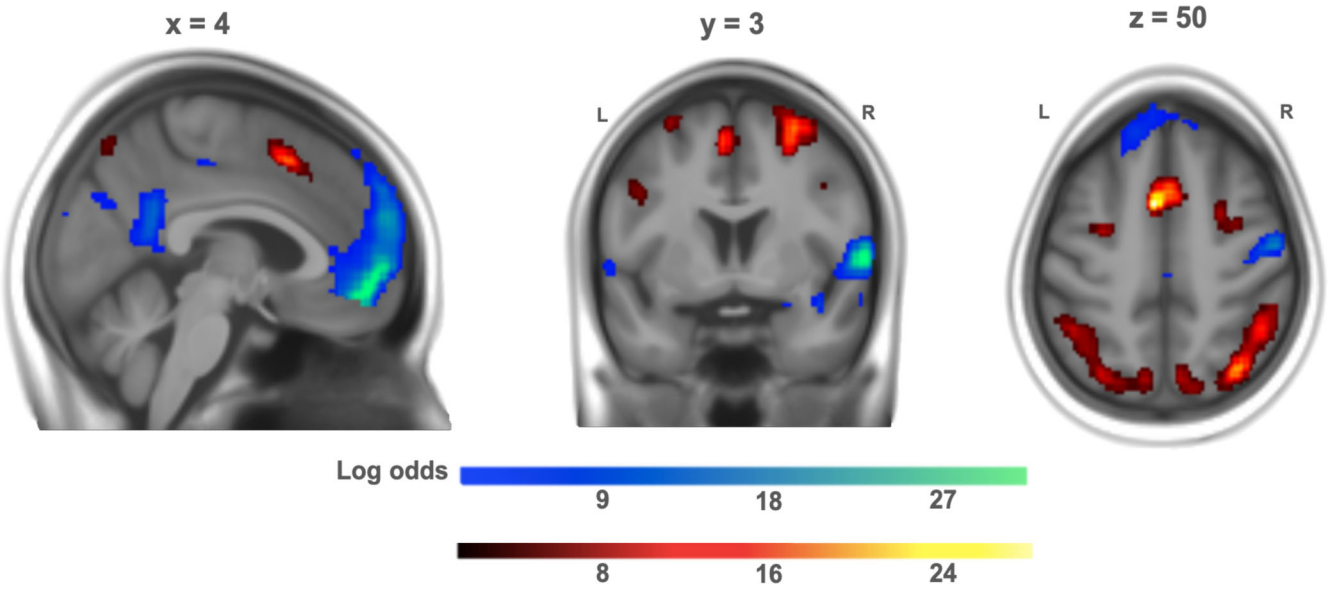

FIGURE 4 Neural correlates of pupil size between conditions. Hot colors: blood oxygen level dependent (BOLD) activity positively correlated with pupil size. Cold colors: BOLD activity negatively correlated with pupil size ($d = 0.2$, logBF >3). L = left, R = right

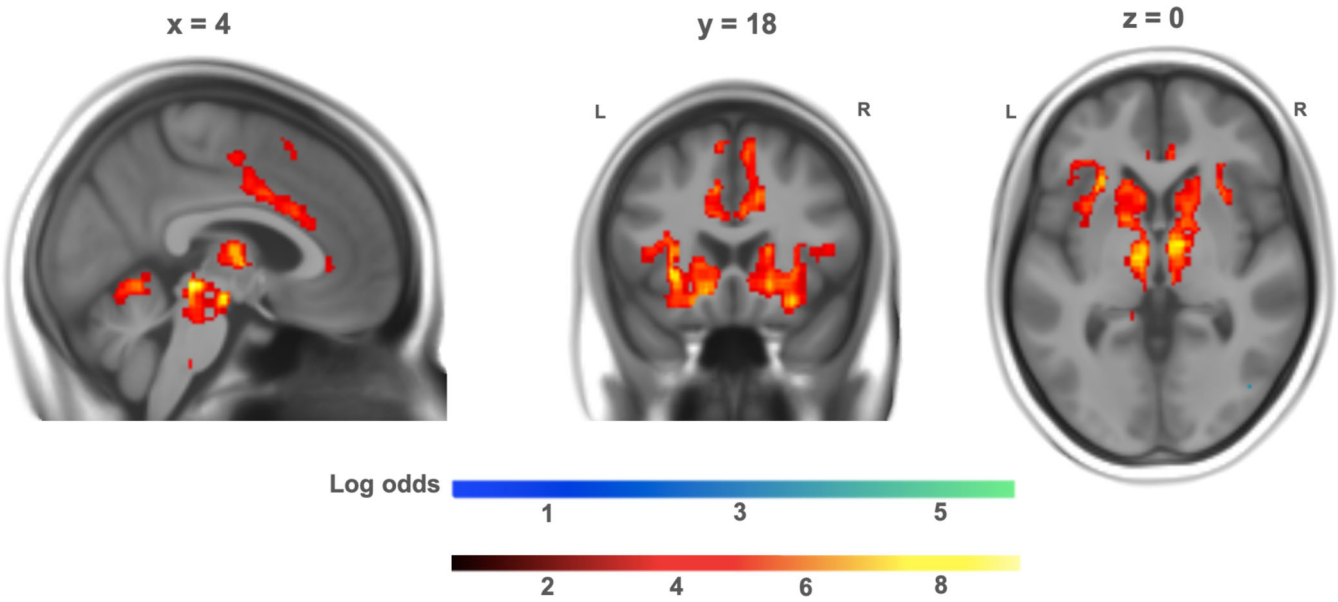

FIGURE 5 Neural correlates of pupil change within conditions. Hot colors: blood oxygen level dependent (BOLD) activity positively correlated with pupil change. Cold colors: BOLD activity negatively correlated with pupil change ($d = 0.5$, logBF >3). L = left, R = right

block was subtracted from the pupil change value in the corresponding 1 s time bins.

If the first analysis would show the salience network but not the second one, this would be evidence for confounding mean differences per block. If the second analysis would show the salience network but not the first, it would be evidence that this network correlates with dynamic within-block fluctuations. We could observe the latter pattern of results (see Figures S10 and S11 in the Supplement), as the second analyses revealed activation in the bilateral insula, caudate, thalamus, orbital inferior frontal gyrus, anterior and middle cingulate cortex ($d = 0.5$, logBF >3). These clusters (Table S6 in the Supplement) showed a strong overlap with those of our main analysis of pupil change within conditions (Figure 4). This supports the notion that it is not the mean pupil change between conditions, but rather pupil change within (i.e., during) the conditions that drives this result.

To further examine if there was an effect of condition on the strength of the salience network adherence to the pupil change dynamics, we performed an ANOVA testing for the main effect of condition and a conjunction analysis with the three N-back conditions, as well as all four conditions. In the ANOVA (main effect), we did not observe relevant activity at the set thresholds (voxelwise $p_{FWE} < .001$). To evaluate whether we missed potentially relevant clusters

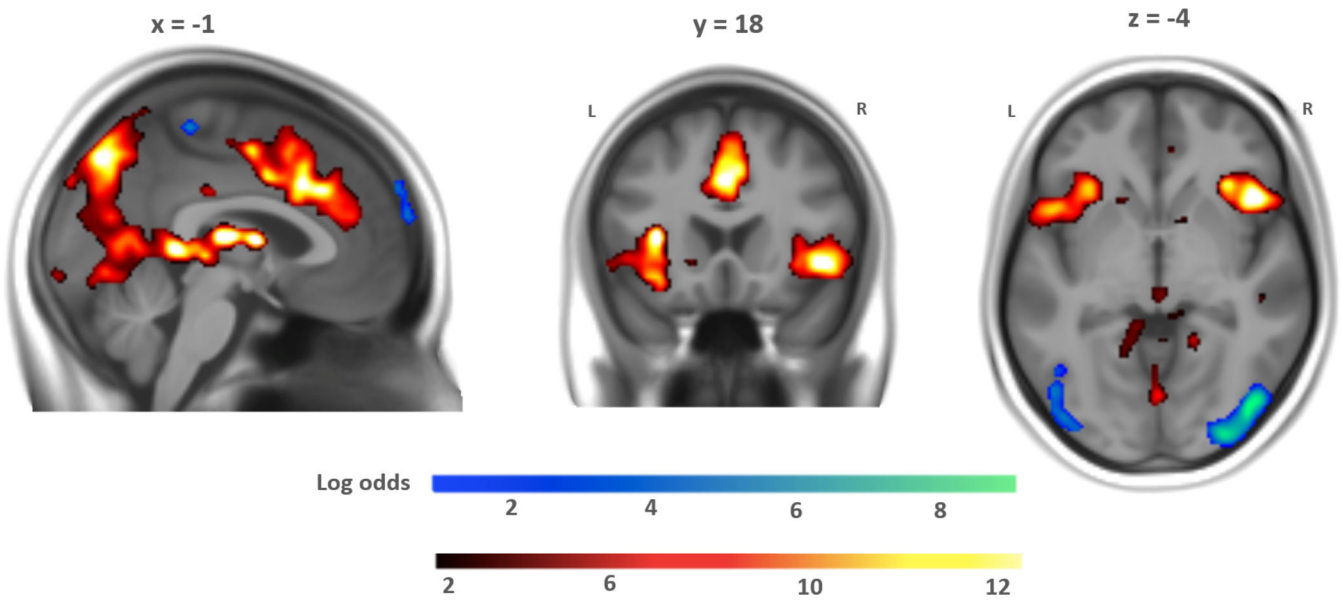

FIGURE 6 Neural correlates of pupil peak (maximum pupil size value in search window) per trial. Hot colors: blood oxygen level dependent (BOLD) activity positively correlated with pupil peaks per trial. Cold colors: BOLD activity negatively correlated with pupil peaks per trial (d = 0.2, logBF >3). L = left, R = right

due to an overly conservative threshold for a comparison among conditions (as opposed to a test against 0 as in the previous tests), we reduced the threshold to a frequentist voxelwise $p_{FWE} < .05$, $k > 50$. At this lower threshold, we could observe eight clusters as listed in Table S7. The activation was mainly evident in the bilateral caudate, middle and posterior cingulate gyrus, inferior frontal gyrus, in the middle frontal gyrus ($p_{FWE} < .05$) (Figure S12 in the Supplement). The conjunction analysis encompassing the parametric modulation of mean pupil change of three conditions requiring a response (0-back, 1-back, and 2-back) showed activation in the bilateral insula, SMA and the inferior frontal gyrus and middle cingulate gyrus in the right hemisphere at this lower threshold (voxelwise $p_{FWE} < .05$, $k > 30$) (Figure S13 in the Supplement). A conjunction analysis of all four conditions (including fixation) only revealed similar activity at a low, uncorrected threshold (uncorrected $p < .001$) (Figure S14 in the Supplement).

The second level GLM of the pupil peak values per trial revealed very strong evidence for positively correlated BOLD activity mainly in the salience network (bilateral insula, dACC), thalamus, and SMA, $d = 0.2$, logBF >3 (Figure 6). The negative contrast revealed strong evidence for corresponding negative correlations mainly within the occipital lobe, $d = 0.2$, logBF >3. For a detailed listing of these clusters, see Table S8 in the Supplement.

3.3.3 | Conjunction: Neural correlates of pupil size and pupil change

We overlaid the contrasts from the analysis on pupil size between conditions and of pupil change within conditions to examine the regional overlap (Figure 7). This conjunction analysis revealed activity in the dACC and bilateral insula for both contrasts. Interestingly, this activity was almost completely nonoverlapping, but rather in adjacent subregions. The between condition pupil size-FPN network revealed slightly more dorsally located clusters in the dACC/SMA, for instance, whereas the within condition pupil change-salience network revealed a more ventrally located cluster just above the corpus callosum with associated activity in the midbrain/brainstem, thalamus, and basal ganglia.

However, this apparent separation disappears at more lenient, frequentist thresholds ($p_{FWE.voxel} < .05$ and $k > 100$), with clusters of activity in dACC and bilateral anterior insula becoming overlapping (Figure S15 in the Supplement).

4 | DISCUSSION

In this study, we investigated the relationship between pupil fluctuations and associated BOLD correlates during working memory processing in healthy humans. For this purpose, participants performed an N-back fMRI task while their pupil size was recorded simultaneously at a high sampling rate. To couple pupillometry with our fMRI analysis, we quantified pupil fluctuations in two ways: (a) as differences of mean pupil size *between* the N-back conditions that were characterized by systematically varying working memory load levels, and (b) as pupil change *within* these conditions. Eventually, these extracted pupil size and pupil change measures were entered into separate first level GLMs of the fMRI BOLD time series.

As hypothesized, our results provided strong evidence for an increase in pupil size with increasing working memory load, confirming a robust interrelation between pupil size and the cognitive effort that was encoded in the experimentally controlled levels of working

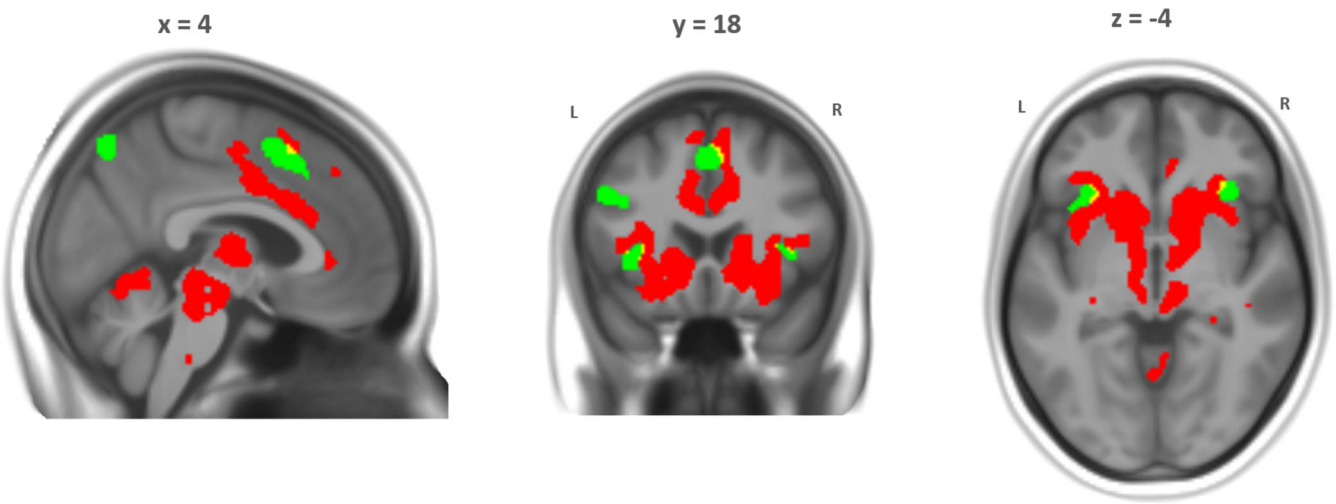

FIGURE 7 Regional overlap (in yellow) of neural correlates of pupil size *between* condition (in green; $d = 0.2$, logBF >3) and pupil change *within* condition (in red; $d = 0.5$, logBF >3)

memory load. This aligns well with previous pupillometry reports (Robison & Unsworth, 2019; Unsworth & Robison, 2018) and our behavioral measures that reflected increasing task difficulty with increasing working memory load, as indicated by typical stepwise RT and accuracy profiles. These behavioral results represent an important validation that at the group level, varying difficulty levels could be successfully induced.

Analysis of BOLD activity linked to pupil size differences *between* conditions yielded very strong evidence for activation of the bilateral FPN including the dorsolateral PFC, ventrolateral PFC, posterior parietal lobule, cerebellum and bilateral insula, albeit a small effect size. Considering previous studies of neural activation during working memory processing (Mencarelli et al., 2019; Owen et al., 2005; Rottschy et al., 2012), our findings related to pupil size *between* conditions were in line with the working memory network gained meta-analytically from 189 studies that revealed a strong, consistent bilateral activation of the FPN encompassing the inferior frontal gyrus, bilateral insula, SMA, superior frontal gyrus, and superior parietal lobule (Rottschy et al., 2012). In the inverse contrast, we observed the default mode network (DMN), the typical task negative network (Raichle, 2015). The results so far support the understanding that pupil size averaged per condition is robustly reflecting the current working memory load at the subject level, similar to analyses that directly model the gradual working memory recruitment of the FPN and DMN (Di, Zhang, & Biswal, 2020).

The question that guided our further analyses, however, was how this can be integrated with the literature on correlations between pupil dilation and the salience network (Leuchs et al., 2017; Schneider et al., 2016, 2018). We addressed this topic by focusing on the neural correlates of mean pupil change *within* conditions and observed very strong evidence with a medium effect size for a positive correlation of pupil change with the activity level of the salience network. We should add that this network, beyond its typical insular and dACC hubs, also relays on the arousal system, such as the thalamus and the posterior cingulate (Menon & Uddin, 2010). This correlation between pupil change (first-order derivative of pupil size) and the salience network was largely independent of the working memory load level, as our secondary analyses revealed practically equivalent maps of the salience network in 0-back, 1-back, and 2-back conditions (Figure S16 in the Supplement) with minor differential effects between these conditions. When examining the peak voxel contrast estimates the activation in the bilateral caudate, for example, was mostly driven by the 2-back condition, possibly pointing toward a certain threshold of task complexity that triggers involvement of the caudate only at the most difficult stage (Figure S17 in the Supplement). Thus, we suggest that cognitive load, or simple motor responding, does affect the correlations between pupil change in more peripheral regions of the salience network but not its core regions.

However, it is important to point out that these two distinct subprocesses of working memory also showed a regional overlap and share parts of the activation patterns mainly in the bilateral insula, dACC, even though the peaks were adjacent and largely non-overlapping within these regions. This conjunction of FPN and salience regions supports the notion that salience network regions, anterior insula and dACC, are involved in both processes: salience detection and cognitive demand. The salience network, and particularly the insula, integrates cognitive information and acts as a switch between large-scale networks to facilitate access to attention and working memory (Menon & Uddin, 2010). Furthermore, the anterior insula and the dACC exhibit a close functional relationship and are fundamental for effort related processes (Medford & Critchley, 2010). In a wide range of cognitive tasks, including the N-back task, a coactivation of the salience network and the PFC is very common (Kurth, Zilles, Fox, Laird, & Eickhoff, 2010; Menon, 2011). Our results, based on the simultaneous measurement of pupillometry and fMRI, point toward a physiological upregulation when a target stimulus is detected in a high demand condition and a response is required, through connecting the insula and dACC with arousal-related regions

(brainstem/midbrain, thalamus, and basal ganglia). According to the adaptive gain theory, the LC receives top-down task-related information from high-level structures and anatomical studies have shown cortical projections from the dACC to the LC in primates (Aston-Jones & Cohen, 2005). The coactivation pattern in our results can be interpreted as a sustained restimulation of the FPN by the salience network, as it is able to lead resources to the FPN. At the same time, the FPN holds the task-relevant information leading to a potentially stronger interconnection between these two networks.

The fixation condition showed a nonintuitive correlation pattern at first glance: here pupil change revealed a correlation with DMN midline hubs and some overlap with the bilateral dorsal ACC (Figure S16 in the Supplement). The salience network activity was less pronounced compared to positive correlations of pupil change with solely salience network areas during the resting state (i.e., unconstrained cognition) (Schneider et al., 2016), and compared to the 0-back, 1-back, and 2-back conditions in our study. One explanation for the salience network being only weakly coupled with pupil change in the fixation condition might be the lack of salient stimuli and/or goal-directed motor responses during that block. In turn, one reason for the appearance of the DMN may be the low cognitive demand of the fixation condition (passive viewing of the same repeating stimuli), which is in contrast to the cognitively more demanding N-back conditions that decrease the DMN "tonically" with less volatility and responsivity to single stimuli. A strong recruitment of the salience network in parallel with pupil dilation seems to occur either at rest (Schneider et al., 2016) when large, low frequency fluctuations are present spontaneously, or in a cognitive context above a cognitive demand threshold that requires actual redistribution of resources from the DMN to FPN. The observed common DMN and salience network recruitment resembles other examples of transient positive coupling between the DMN and other high control networks. Piccoli et al. (2015) reported that during specific subphases of a working memory task—encoding and retrieval—the DMN and the FPN coupled positively, whereas during the maintenance phase with no visual input these networks remained anticorrelated (Piccoli et al., 2015). The salience network plays an important role in promoting such switches (Menon, 2011, 2015; Menon & Uddin, 2010), and our *within* condition results demonstrate that salient stimuli trigger its activity to uphold the functional segregation between the DMN and the antagonistic FPN.

Further, we interpret the differences *between* and *within* condition correlations with pupil size and pupil change as reflecting differences between tonic versus phasic arousal, respectively: The correlation of mean pupil size and activity in the FPN could relate to a tonic pupillary response that increases as the task becomes more challenging. In addition, and occurring concurrently, the active N-back conditions contain target stimuli conceivably triggering a phasic response that correlates with the salience network independent of working memory load.

Both the tonic and phasic pupillary arousal states could be attributed to the LC norepinephrine (NE) system (Aston-Jones & Cohen, 2005; Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010). The LC is a neuromodulatory nucleus in the brainstem that is responsible for most the NE released in the brain, and it has widespread projections throughout the neocortex including frontal–parietal areas. Pupil dilations related to cognitive processing are thought to result from an inhibitory effect on the parasympathetic oculomotor complex by release of NE from the LC (Szabadi, 2013). A possible explanation for our observation might be that the task demand results in an increased firing rate of LC neurons, which leads to an enlargement of the pupil diameter and facilitation of working memory processing in the PFC areas, which again is interconnected with the LC constituting a reciprocal relationship (Alnaes et al., 2014; Arnsten, Wang, & Paspalas, 2012; Mather et al., 2020; Sara & Bouret, 2012). To date, there are no existing studies explicitly relating LC neuronal activity to working memory, but neuropharmacological studies provide evidence of the essential role of NE release for executive functioning (Arnsten et al., 2012; Ramos & Arnsten, 2007). Hitherto, the increase in pupil size during working memory was associated with task-evoked phasic arousal, arguing that attention was constantly allocated in order to actively maintain items in working memory (Unsworth & Robison, 2018). We speculate that the LC tonic activity might be responsible for the general increase in the overall pupil size *between* conditions and the LC phasic activity may be related to the pupil change *within* conditions generated by the target stimuli. In the event-related analyses (Figures 3 and 6), we showed that the within condition pupil responses were specifically related to the trials, which are primarily affected by stimulus type (target vs. non-target) as larger pupil dilations were associated with target trials and elicited activation in the typical salience brain regions. Interestingly, the pupil dilation in response to target trials was larger for the trials with lower cognitive load. This is most likely a consequence of the larger mean pupil size in the higher cognitive load conditions (Peysakhovich, Vachon, & Dehais, 2017).

This raises the question of whether these stimulus type driven modulations are associated with stimulus saliency or effort allocation. In our N-back task, the target and non-target trials were not distinguishable by visual features alone. The participants needed to constantly update their information in mind and then identify the target solely by correctly memorizing the preceding trials (and in the case of the 0-back condition remembering one specific stimulus), meaning that the identification of the salience of targets required task-engagement and effort allocation toward the stimulus. Research on primates has shown that the phasic response is not particularly linked to specific sensory attributes of a stimulus, but rather to task-relevant events (Aston-Jones & Cohen, 2005). Following this line of thought, it is possible that the effort allocation precedes the experienced salience of the target, and the resulting correlation with the salience brain networks is a product of both processes (Engström, Landtblom, & Karlsson, 2013).

The relationship of pupil fluctuations and neural activity is probably not exclusively dependent on the LC and the general noradrenergic tone controlled by it. Electrophysiological research in rhesus monkeys has pointed toward a similar relationship of pupil temporal dynamics and the inferior and superior colliculus in the mesencephalon. Additionally, neural activity in the dACC could also be aligned in time with changes in pupil diameter, reflecting underlying changes in arousal (Joshi, Li, Kalwani, & Gold, 2016).

Murphy et al. (2014) observed a very similar pattern of activation as they also found positive correlations of pupil diameter with activity in ACC, insula, and the thalamus in an oddball task. Moreover, they could show that pupil diameter was positively correlated with BOLD activity in the rostral LC (peri-LC), providing the first fMRI evidence supporting the notion that the pupil diameter can be used as indirect measure of LC activity (Murphy et al., 2014). In our analyses of pupil change *within* conditions, we also observed activity in the brainstem in areas that could encompass the LC, but our resolution and preprocessing was not optimized for brainstem analyses.

Although the N-back task is well-established and has been one of the most commonly used experimental paradigms for exploring the neural basis of working memory and executive functioning (Lamichhane, Westbrook, Cole, & Braver, 2020), there are a few methodological considerations with respect to our interpretations. The blocks of the N-back task utilized in this study did not follow a randomized order, which means that theoretically the fixed order could have an influence on the results. Nevertheless, as each condition was present once in the first half and once in the second half of the task, and as no tiring effect was detected in the pupil data, we assume that the influence of the design limitation was marginal (for analysis, see section 2.3 in the Supplement). Another restraint may lie in the conditions of the task itself, which is noticeable in the accuracy rates that showed overall a very high level of correct responses. Although we observed large differences in accuracy between 1-back and 2-back, and 0-back and 2-back, we did not observe a difference between the 0-back and 1-back condition in accuracy rates. This is probably due to a ceiling effect, with similar patterns observed in healthy subjects in previous work (Hur, Iordan, Dolcos, & Berenbaum, 2017; Jacola et al., 2014). These authors have proposed that RTs represent a more meaningful readout for the N-back task. In our study, we could observe a difference between all conditions regarding that measure. The condition with the maximum working memory load was 2-back, and conditions with higher load are generally feasible in healthy subjects. The reason for not including a 3-back condition is that our task is part of a larger study on psychiatric patients, some of which have mood disorders with cognitive impairments, and healthy participants. However, when taking our pupillary, behavioral and the neural readouts into account, we can safely claim that the working memory manipulation was successful, similar to previous work that did not include a 3-back condition (Alonso-Lana et al., 2016; Dores et al., 2017; Peysakhovich et al., 2017). Nevertheless, future research could incorporate conditions with higher load in order to be able to observe a potential inverted U-shaped pursuant to the Yerkes–Dodson law (Yerkes & Dodson, 1908). Prior research on this has shown, that pupillary dilation during a working memory task increased until it reached an asymptote at around four to five items held in mental storage (Robison & Unsworth, 2019; Unsworth & Robison, 2018). This could be of potential interest as previous research has related this pattern to the influence of NE on PFC functioning. In their work, the NE release was dose dependent and also followed an inverted U relationship, suggesting that performance increases with physiological or mental arousal, but only up to a certain point until it reaches a plateau before starting to decline (Aston-Jones & Cohen, 2005; Sara & Bouret, 2012).

To summarize, our findings suggest that fMRI with simultaneous measurement of pupil parameters constitutes a valuable experimental setup to decipher cognitive processes related to working memory load itself versus the immediate salience of the presented stimuli. This distinction could be specifically relevant for patients with psychiatric disorders. Cognitive impairment and in particular, working memory deficits manifest in a wide range of psychiatric disorders both of the affective and psychotic spectrum (Snyder, 2013). It has thus been proposed as a transdiagnostic endophenotype or risk factor (Nolen-Hoeksema & Watkins, 2011). Similarly, the salience network has been identified as critical to psychiatric disease susceptibility (Goodkind et al., 2015) across the affective and psychosis spectrum, and as such, combined, sensitive tools for studying working memory processes and their link to salience activity are particularly relevant. To this notion we add, that two working memory subprocesses related to cognitive load and salience could be distinguished by parallel fMRI and pupillometry, which could help develop a more valid biological characterization of working memory processes and deficits.

5 | CONCLUSION

Incorporating pupillometry in fMRI measurements during a working memory task allowed differentiation between working memory load effects and effects of the salience of the presented stimuli. We demonstrated, that the mean pupil size *between* condition was related to the FPN and that pupil change *within* conditions was associated with activity in the salience network, independently from working memory load. This combination of pupil and fMRI parameters may constitute an effective tool for disentangling working memory subprocesses that could be relevant for a range of psychopathological conditions.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Julia Fietz: Methodology, software, formal analysis, data curation, writing - original draft, writing—review and editing, visualization. **Dorothee Pöhlchen**: Software, formal analysis, writing—review and

editing. **Florian P. Binder**: Software, writing—review and editing. **BeCOME Working Group**: Investigation, resources, project administration. **Michael Czisch**: Conceptualization, methodology, resources, writing—review and editing. **Philipp G. Sämann**: Conceptualization, methodology, software, resources, writing—original draft, writing—review and editing. **Victor I. Spoormaker**: Conceptualization, methodology, resources, writing—original draft, writing—review and editing, supervision.

DATA AVAILABILITY STATEMENT

The data were collected as part of the ongoing BeCOME project (Brückl et al., 2020) at the Max Planck Institute of Psychiatry in Munich, Germany. The data that support the findings of this study are available from the corresponding author upon reasonable request. The unthresholded statistical maps from the second level analyses are available on NeuroVault (https://identifiers.org/neurovault.collection:11191).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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4

Paper 2 | Data-driven pupillometric response profiles as transdiagnostic biomarkers in affective and anxiety disorders

4.1 Summary

The goal of the second study was to test whether individual pupillometric response profiles can function as indication ("biomarker") for neurocognitive deficits while cutting across diagnoses of psychiatric disorders. As discussed before neurocognitive deficits constitute a general common symptom in psychopathology (Caspi et al., 2014), which makes it a substantial transdiagnostic factor. For the analysis latent class growth modelling, a data-driven, regression-based clustering approach was used in order to find potential groups based on individual pupil size patterns during working memory processing. The data were acquired as part of the BeCOME study, but this time using the full spectrum reaching from healthy control participants to (medication-free) patients with psychiatric diagnoses. The analysis included participants i) who had neither current, nor history of a psychiatric disorder, and ii) participants with mainly affective and anxiety disorders and its common comorbidities such as post-traumatic stress disorder or obsessive-compulsive disorder.

The clustering produced two distinct pupil response profiles: (1) a group exhibiting a gradual increase in pupil size with increasing cognitive load (reactive group), and (2) another displaying an attenuated pupil response (non-reactive group). These profiles were significantly related to neurocognitive performance in executive functioning and sustained attention. Individuals who were more likely to belong to the reactive pupil response profile performed better on these neurocognitive tests. Additionally, the resulting clusters were biologically validated in fMRI

through adding the groups mean pupil size scores as parametric modulation to the GLM. The results indicated that the data-driven clusters also revealed differential neural correlates in the N-back task: The reactive pupil response profile was associated with more activity in the thalamus and less deactivation of the limbic system, potentially reflecting a better arousal upregulation. The non-reactive pupil response profile showed stronger neural correlates in the FPN regions.

This study went beyond behavioral readouts for neurocognition and across rigid diagnostic criteria. Through applying a data-driven approach distinct pupil response profiles related to neurocognitive performance were deciphered, which correlated with differential patterns of arousal on a brain activity level. Based on these findings, pupil measurements have the potential to serve as a highly sensitive and precise readout, and a psychophysiological biomarker for early detection of neurocognitive deficits mainly within executive functioning.

4.2 Contributions and reference

The study "Data-driven pupil response profiles as transdiagnostic readouts for the detection of neurocognitive functioning in affective and anxiety disorders" was published in Biological Psychiatry: Cognitive Neuroscience and Neuroimaging in 2023.

The project was conducted under the supervision of VS. The concept of the study was designed by VS, PS, MC, AKB, TB and the BeCOME study team. Data were acquired by the BeCOME study team. The conception of the data analysis pipeline was done by JF and VS. The data analysis was performed by JF. The data were interpreted by JF, VS, PS, DP. All authors critically revised the manuscript written by JF and VS.

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Biological Psychiatry: CNNI

Archival Report

Data-Driven Pupil Response Profiles as Transdiagnostic Readouts for the Detection of Neurocognitive Functioning in Affective and Anxiety Disorders

Julia Fietz, Dorothee Pöhlchen, BeCOME working group, Tanja M. Brückl, Anna-Katharine Brem, Frank Padberg, Michael Czisch, Philipp G. Sämann, and Victor I. Spoormaker

ABSTRACT

BACKGROUND: Neurocognitive functioning is a relevant transdiagnostic dimension in psychiatry. As pupil size dynamics track cognitive load during a working memory task, we aimed to explore if this parameter allows identification of psychophysiological subtypes in healthy participants and patients with affective and anxiety disorders.

METHODS: Our sample consisted of 226 participants who completed the n-back task during simultaneous functional magnetic resonance imaging and pupillometry measurements. We used latent class growth modeling to identify clusters based on pupil size in response to cognitive load. In a second step, these clusters were compared on affective and anxiety symptom levels, performance in neurocognitive tests, and functional magnetic resonance imaging activity.

RESULTS: The clustering analysis resulted in two distinct pupil response profiles: one with a stepwise increasing pupil size with increasing cognitive load (reactive group) and one with a constant pupil size across conditions (nonreactive group). A larger increase in pupil size was significantly associated with better performance in neurocognitive tests in executive functioning and sustained attention. Statistical maps of parametric modulation of pupil size during the n-back task showed the frontoparietal network in the positive contrast and the default mode network in the negative contrast. The pupil response profile of the reactive group was associated with more thalamic activity, likely reflecting better arousal upregulation and less deactivation of the limbic system.

CONCLUSIONS: Pupil measurements have the potential to serve as a highly sensitive psychophysiological readout for detection of neurocognitive deficits in the core domain of executive functioning, adding to the development of valid transdiagnostic constructs in psychiatry.

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Neurocognitive malfunctioning is a transdiagnostic phenomenon (1,2) associated with psychopathology across multiple disorders including anxiety disorders (3), mood disorders (4), obsessive-compulsive disorder (5), schizophrenia (6), anorexia nervosa (7), bulimia nervosa (8), substance use disorders (9), and several personality disorders (10). Similarly, when moving toward a more dimensional framework, neurocognitive malfunctioning is linked to a whole spectrum of psychopathological syndromes, such as rumination (11), dissociation (12), and schizotypy (13), in addition to perceived subjective burden and illness chronicity (14). Furthermore, baseline neurocognitive functioning has been found to be predictive of treatment response in pharmacotherapy and psychotherapy (15).

One advantage of neurocognitive tests is that on top of the objective behavioral measures (1), physiological parameters of cognitive processing can readily be added to provide additional, more in-depth understanding of the brain-behavior relationship. Pupillometry is ideally suited to examine task-related brain mechanisms as it provides information of underlying physiological processes with high precision (16). The pupil dilates or constricts in response to arousal and mental effort through the modulation of several brainstem and subcortical regions including the superior colliculus, the cholinergic basal forebrain (17), and the locus coeruleus (LC), a nucleus in the brainstem involved in physiological arousal and cognitive functioning and the brain's main noradrenergic output center (18). The interconnection between the noradrenergic (NE) activity of the LC and the pupil has been established in numerous anatomical and physiological studies in humans (19,20), primates (17,21), and rodents (22,23). Given the importance of the LC-NE system for regulating attentional status, it has been postulated that pupil-linked individual differences in working memory (WM) may reflect regulation and dysregulation of the LC-NE system (24).

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In WM paradigms, pupil size robustly depends on cognitive load (25–27). In our previous work in healthy human participants, we could confirm this notion as pupil size increased with cognitive load across conditions in parallel with activity in the frontoparietal network (FPN) (25), the typically observed WM large-scale brain network (28). Individual differences in WM have been shown to predict performance in a variety of other cognitive domains (24), such as learning (29) and fluid reasoning (30), making it a core topic of interest for investigation in the general population as well as in psychiatric samples (31). This notion is further supported by converging evidence for a link between pupillary motility alterations and depression during various processes (32). For example, research has shown that individuals with depression or elevated risk of depression had higher pupillary reactivity toward stimuli with negative valence (33). Further, rumination, a pattern of repetitive and intrusive negative thinking, was associated with greater baseline pupil dilation in depressed adolescents (34). Interestingly, change in rumination following a cognitive training program was predicted by pupillary oscillations, reflecting task engagement at the beginning of the training (35). Moreover, pupil dilation representing reward anticipatory arousal has been found to negatively correlate with depressive symptom load, i.e., the more acute depressive symptoms, the lower the pupil dilation during the anticipation of a reward (36).

However, it is largely unknown whether task-evoked pupil size constitutes a valid physiological readout for neurocognitive functioning in a dimensional, cross-diagnostic approach. In this study, our aim was to identify data-driven pupil response profiles during WM processing in a heterogeneous sample including healthy adults as well as unmedicated patients. For this purpose, we simultaneously recorded pupil size and functional magnetic resonance imaging (fMRI) while participants performed the well-established n-back task (37,38). We hypothesized that individual pupil response profiles during WM would be linked to performance in neurocognitive tests of various cognitive domains and symptom severity and would show differential blood oxygen level–dependent (BOLD) activity patterns in the FPN and arousal (salience) networks. Additionally, we were interested in how mental disorders on the affective and anxiety spectrum modulated these effects.

METHODS AND MATERIALS

Participants

Data for the presented analyses were obtained from the ongoing Biological Classification of Mental Disorders (BeCOME) study at the Max Planck Institute of Psychiatry in Munich, Germany (ClinicalTrials.gov: NCT03984084). Local ethics approval was obtained for this study. Unmedicated patients with mainly Axis I disorders and healthy control participants were included. Further recruitment strategies and inclusion criteria have been previously described in detail elsewhere (39). The BeCOME study protocol was in accordance with the Declaration of Helsinki. All participants provided written informed consent after the study protocol had been fully explained and were compensated for their participation (39). The sample for this project included all participants with combined fMRI/pupillometry n-back measurements obtained up until January 14, 2020. Of the 248 recruited participants (166 women, mean [SD] age = 35.0 [12.2] years) who took part in the n-back task, 226 participants (152 women, mean [SD] age = 34.7 [11.9] years) had available eye tracking data, which served as basis for the clustering approach using latent class growth modeling (LCGM) (40). For all subsequent analyses, we excluded 5 patients who required medication between the screening and study day 1 (and thus reported the intake of psychopharmacological medication on study day 1), leading to a sample size of 221 participants (151 women, mean age = 34.58 [11.82] years). A more detailed description of sample composition is presented in 1.1 in the Supplement.

Psychometric Instruments

During the inclusion visit, which took place 1 to 2 weeks before the first of 2 study days, all participants underwent a standardized diagnostic interview (DIA-X/Munich Composite International Diagnostic Interview [M-CIDI]) (41) assessing the diagnosis of mental disorders according to DSM-IV including information on onset, duration, and severity (39). For the measurement of depressive symptoms and negative affectivity (42), we used the Beck Depression Inventory-II (BDI-II) (43) and the State-Trait Anxiety Inventory (44), respectively. Anhedonia was assessed using the BDI-II anhedonic subscore consisting of the following items: loss of pleasure (item no. 4), loss of interest (item no. 12), loss of energy (item no. 15), and loss of interest in sex (item no. 21) (45). The questionnaires were administered on the first study day.

Neurocognitive Assessment

The neurocognitive testing session was scheduled on the first study day at the same time of day (11 AM) and lasted 1 hour. The order of the test administration was identical in all participants, and they received written and verbal instructions before each test (39). We administered tests for the following domains: cognitive flexibility (46), inhibitory control (46), sustained attention (47), episodic memory (48), word fluency (48), and crystallized intelligence (49). A detailed description of each test can be found in 1.2 in the Supplement.

fMRI/Pupillometry n-Back Task

The fMRI n-back task was part of the first scanning session on the first study day (39). Before entering the scanner, all participants received task instructions and performed a short, standardized, computer-based training. After making sure that the task was fully understood, the participants were placed in the MRI scanner.

In the n-back task, a sequence of capital letters was displayed on the screen, and participants were asked to respond when the current letter matched the one from “n” steps before. The task included 4 conditions: fixation only (without any response), 0-back (just responding to the target letter), 1-back, and 2-back. Each condition was presented twice within a block design while fMRI and pupillometry were recorded simultaneously. For assessment of task performance, we used individual mean reaction time and mean accuracy rates across respective conditions. A more detailed description of the task

has been published elsewhere (25) and is included in 1.3 in the Supplement.

Pupillometry

Pupil size of the participant's right eye was recorded during the n-back task at a sampling rate of 250 Hz with an MRI-compatible eye tracker (EyeLink 1000 Plus; SR Research). The data were processed and analyzed in MATLAB (version 2021b; The MathWorks, Inc.). Missing data related to eye blinks were linearly interpolated between the last saccade before blink onset and the first saccade after blink offset. Additionally, pupil size data were smoothed by replacing values with the mean of a 200-ms sliding window and *z*-transformed to control for variability across participants. We calculated individual mean pupil size values per task block (2 blocks per condition, 4 conditions).

Statistical Analyses

LCGM was used to identify latent classes of pupil responses (40). This is a data-driven modeling technique that detects heterogeneity in time series data. Eventually, it clusters individuals based on their growth trajectories, which are defined by growth parameters such as intercept and slope (50). The LCGM analysis was performed in R (version 4.2.0; R Foundation for Statistical Computing) using the *flexmix* package (51). Individual mean pupil size values per condition of the n-back task were entered into the algorithm. To identify the optimal number of classes, models with increasing number of latent classes (from 1 to 10) were fitted to the data, and the best-fitting model based on the Bayesian information criterion was selected. The variance estimate for Gaussian components was constrained to be equal, and the clustering model was run with 200 repetitions to achieve a stable cluster solution. To validate the stability of the clustering, we used the holdout method as an internal validation approach. We drew random subsamples while leaving out 30% of participants and repeated this analysis 5 times. We reviewed whether it led each time to the same cluster solution so that we confidently could use the original one for further analysis.

To investigate associations between the resulting pupil response profiles and psychopathology, neurocognitive functioning, and behavioral parameters in the n-back task, we estimated linear regression models in MATLAB (*fitlm*). The models were adjusted for gender, age, and years of education. Bonferroni-corrected significance levels were used to account for multiple testing (.05/2 = .025 for the psychometric instruments, .05/6 = .008 for neurocognitive functioning, .05/2 = .025 for behavioral parameters in the n-back task).

For additional analyses, we split the sample and investigated healthy control participants and patients with a DIA-X/M-CIDI ascertained DSM-IV diagnosis and compared their pupil response profile associations using analyses of covariance in R (version 4.2.0). Here, we used a subsample of participants with either no current or past history of mental disorders (healthy control participants, n = 36) and participants who fulfilled the criteria for a full diagnosis related to affective and anxiety disorders, posttraumatic stress disorder, and obsessive-compulsive disorder in the past 4 weeks as verified by the M-CIDI (n = 95) (41). For this exploratory comparison of clinically defined groups, we added age as a standard covariate for psychophysiological research.

To evaluate the accuracy of significant results, we checked the normal distribution of residuals and multicollinearity in our models. We examined model residuals with quantile-quantile plots and additional Kolmogorov-Smirnov tests. If model residuals deviated from normality, we performed additional bootstrapping linear regression models in R (version 4.1.2) using the boot package with 1000 bootstrap replicates. We calculated 95% CIs to verify the significant result.

fMRI Preprocessing and Data Analysis

For fMRI data acquisition, a 3T MRI scanner (Discovery MR750; GE Healthcare) with a 32-channel head coil, covering 40 slices (anterior commissure–posterior commissure orientation of the slices, 96 × 96 matrix, in-plane field of view 24 × 24 cm^2, 3-mm slice thickness, 0.5-mm slice gap, resulting voxel size 2.5 × 2.5 × 3.5 mm^3, echo planar imaging, repetition time 2.5 seconds, echo time 30 ms, acceleration factor 2) was used. Functional data were 176 T2*-weighted echo planar images for the n-back task, where the first 4 volumes were discarded to avoid non–steady-state effects.

For the preprocessing of the functional images, we used the same pipeline as reported previously (25); a detailed description can be found in 1.4 in the Supplement. Essentially, we entered the respective pupil size group means as a parametric modulation to the 8 task blocks in the fMRI analyses and used this modulation for our primary first- and second-level contrasts (see 1.4 in the Supplement for the first- and second-level general linear models).

RESULTS

A description of the sociodemographic variables for 226 participants that were used for the clustering is depicted in Table 1.

Clustering of Pupil Response Profiles

The lowest value of the Bayesian information criterion was found for the two-cluster solution: one cluster with a stepwise increasing pupil size with increasing cognitive load (n = 178, reactive pupil response profile) and one with a constant pupil size across conditions and only a higher value in the most demanding condition (n = 48, nonreactive pupil response profile) (Figure 1A). Rerunning the analysis with randomly selected subsamples resulted all 5 times according to the Bayesian information criterion in the same two-cluster solution with similar patterns of pupil response profiles. An overview of the resulting model-fit indices and a detailed sociodemographic characterization of the clusters can be found in 2.1 in the Supplement. The clusters differed significantly in age, and therefore all subsequent analyses were controlled for this variable.

We derived individual pupil profile (IPP) scores from the resulting clusters by subtracting mean pupil size of fixation from pupil size of 1-back, as the slope between these two conditions provided the maximal differentiation between the clusters. These IPP scores showed a strong correlation with posterior probabilities extracted from the model (ρ = 0.76, $p <$ .001), and a higher IPP corresponded to a stronger reactivity of

Table 1. Sample Characteristics

	Participants Eligible for Analyses, *n* = 226	Healthy Control Participants, *n* = 36	Patients With DSM-IV Diagnosis, *n* = 99
Age, Years, Mean (SD)	34.7 (11.8)	32.4 (10.3)	36.0 (12.9)
Gender, Female, *n*	152	23	69
Years of Education, Mean (SD)	12.2 (1.3)	12.3 (1.2)	12.0 (1.4)
Race/Ethnicity, *n*			
African	1	0	0
Asian	8	1	3
Caucasian	178	30	75
Hispanic/Latin American	3	0	2
East Asian	5	1	3
Unknown	3	0	0
Other	6	0	5
Not indicated	22	4	11
Full DSM-IV Diagnosis, Within Past 4 Weeks, *n*			
Affective disorders	61	0	61
Anxiety-related disorders	73	0	73
PTSD	10	0	10
OCD	21	0	21

The full diagnoses were assessed with the DIA-X/Munich Composite International Diagnostic Interview (41). One participant can have multiple disorders. The ICD-10 codes for the diagnoses are provided in 1.5 in the Supplement.

PTSD, posttraumatic stress disorder; OCD, obsessive-compulsive disorder.

pupil responses. Descriptive statistics of mean pupil size are shown in 2.2 in the Supplement.

Associations of IPP With Self-reported Symptoms, Neurocognitive Performance, and Mental Health Status

No significant associations of IPP with depression (BDI-II, $\beta = -0.591$, $p = .65$), anhedonia ($\beta = -0.062$, $p = .83$), or negative affectivity (State-Trait Anxiety Inventory, $\beta = 0.386$, $p = .74$) symptoms were observed across the groups. The linear regression models revealed that the IPP significantly predicted reaction times in the 0-back condition ($\beta = -0.017$, $p = .006$) and nominally significantly in the 2-back condition ($\beta = -0.025$, $p = .05$), although the latter did not survive the Bonferroni correction (Figure 1B). Neither reaction time in the 1-back condition ($\beta = -0.016$, $p = .15$) nor accuracy rates (0-back: $\beta = -0.003$, $p = .44$; 1-back: $\beta = 0.006$, $p = .18$; 2-back: $\beta = 0.014$, $p = .11$) yielded significant associations with the IPP.

Furthermore, the investigation of associations between IPP and additional neurocognitive tests showed a significant effect for reaction time in cognitive flexibility ($\beta = -94.171$, $p = .0002$), inhibitory control ($\beta = -22.350$, $p = .02$), and performance score in the sustained attention task ($\beta = 13.230$, $p = .006$) (Figure 1C), of which only the result for inhibitory control did not survive Bonferroni correction. Overall, participants with a higher IPP performed better on these neurocognitive measures. Tests within the linguistic domain, such as verbal memory (short term: $\beta = 0.489$, $p = .31$; long term: $\beta = 0.788$, $p = .13$), word fluency ($\beta = 2.235$, $p = .07$), and crystallized intelligence ($\beta = 0.297$, $p = .51$), were not correlated with the IPP.

The assumption of normally distributed residuals was violated for the model with reaction time for the 0-back condition. The CIs from the bootstrapping procedure did not include zero; therefore, we could assume that the effect was significant with $p < .05$. No issues regarding multicollinearity were observed in our regression analysis for all independent variables (variance inflation factor < 2.5). For more details, see 2.3 in the Supplement.

The results of the analysis of how IPP and neurocognitive measures were modulated by diagnostic status (absence vs. presence of DIA-X/M-CIDI ascertained diagnosis) are reported in 2.4 in the Supplement.

Functional MRI

In both groups, reactive and nonreactive, the parametric modulation of mean pupil size per condition was positively correlated with activity mainly in the FPN, which includes the dorsolateral prefrontal cortex (PFC) (superior frontal gyrus, middle frontal gyrus, and supplementary motor area), ventrolateral PFC (inferior frontal gyrus), and inferior parietal lobules, and was negatively correlated mainly with activity in the default mode network (DMN) and cingulate regions, including precuneus, posterior cingulate cortex, dorsomedial PFC, and lateral parietal cortex (angular gyrus) (Figure 2A, B). The interaction contrast comparing the parametric modulation between the two clusters showed higher correlations to pupil size in the FPN (superior and inferior frontal gyrus and posterior parietal lobules) in the nonreactive group compared with the reactive group (Figure 2C), with both correlations being positive. This was accompanied by more negative correlations in the deactivated regions in the nonreactive group, which included the DMN, but also extended to the amygdala, anterior cingulate gyrus, hippocampus, nucleus accumbens, caudate, and thalamus. The latter region showed positive coupling to pupil size in the reactive group and negative coupling in the nonreactive group. The resulting contrast estimates within the mentioned regions and a detailed listing of the resulting clusters are depicted in 2.5 in the Supplement. Additionally, we ran a control analysis excluding participants with excessive motion within the MRI scanner. The results followed a similar pattern and are reported in 2.6 in the Supplement.

DISCUSSION

We used LCGM with individual pupil size values of the different cognitive load conditions from an n-back task and observed two robust clusters of pupil response profiles. The clusters were characterized by a reactive pattern, i.e., pupil size increasing in parallel to increasing WM load and a nonreactive pattern without such a stepwise increase in pupil size. The pupil response profiles were significantly associated with neurocognitive functioning, such as reaction times in 0-back, 2-back, cognitive flexibility, and inhibitory control as well as the performance score in sustained attention, while there were no associations with tests of the linguistic domain or depressive symptoms and negative affectivity. On that basis, pupillometry,

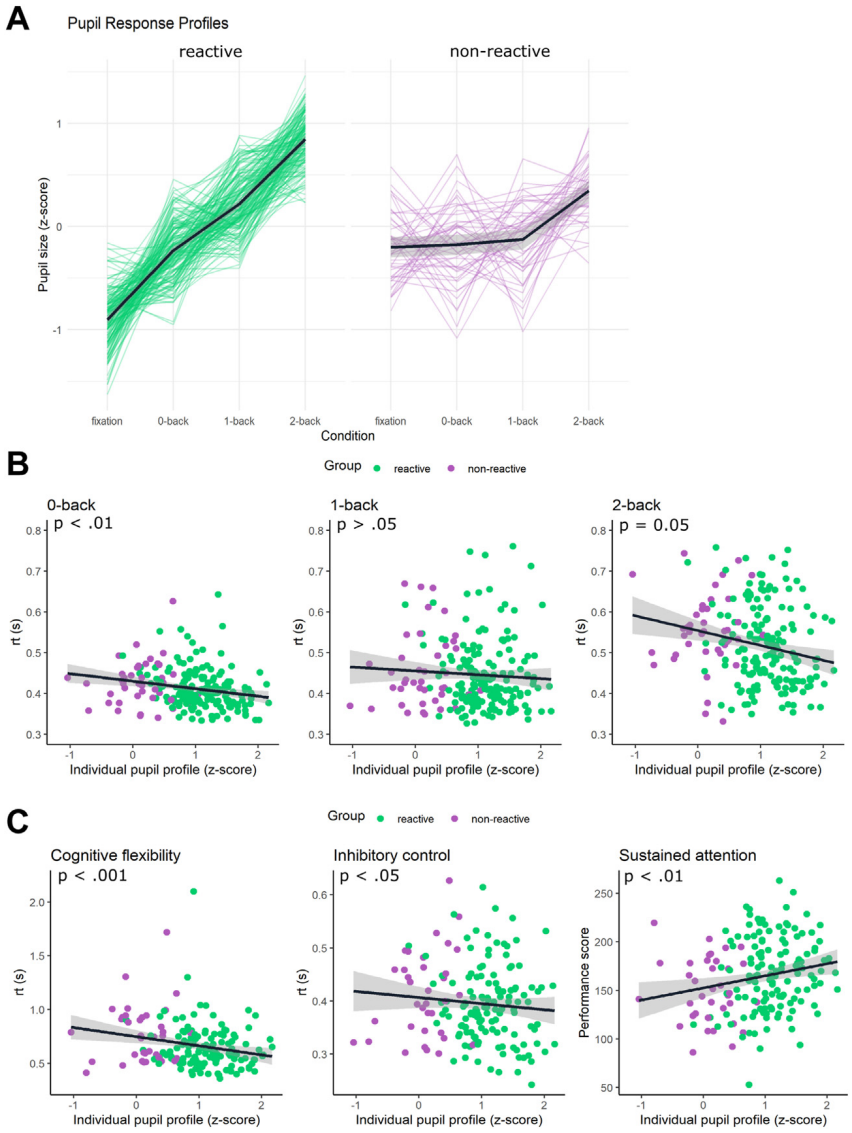

Figure 1. **(A)** Two data-driven clusters from the latent class growth modeling analysis with different individual pupil profiles. **(B)** Correlations between individual pupil profiles and the mean reaction times (rt) for the conditions of the n-back task. **(C)** Correlations between individual pupil profiles and neurocognitive measures. Shaded areas represent 95% CIs. The depicted *p* values are related to the full linear models including covariates. The plots are not corrected for covariates.

and more specifically mean pupil size during WM processing, allowed a stable clustering within a transdiagnostic sample, while behavioral measures of neurocognitive functioning did not (see 2.7 in the Supplement).

Participants with a reactive pupil response profile performed better on neurocognitive tasks, primarily related to sustained attention and processing speed in executive functioning, which is in line with the notion that pupil size could act as a biological, sensitive readout of neurocognition within these domains. Generally, pupil dilation during cognitive tasks is known to increase in relation to increased cognitive demand, which can be reliably observed in the n-back task (52–54). The task-evoked pupil dilation reflects processes related to mental effort and available task-related resources, which can be associated with performance in the given task (55).

Furthermore, the differences in pupil response profiles could also be modulated by arousal states, as most participants are able to upregulate their arousal system during the task at hand to maintain attention and perform accordingly to the instructions. However, a plausible alternative explanation is that a lack of task engagement or motivation has led to a nonreactive pupil response profile.

To examine this more closely, we correlated the IPP with a sum of 3 anhedonia items from the BDI-II and did not observe a correlation. The question is to what extent such items capture task (dis)engagement well, but if we would have found a correlation with anhedonia as a proxy for more general motivation, this would have been an argument in favor of the alternative explanation. Moreover, we observed a correlation between the IPP and neurocognitive measures of sustained attention and executive functioning in another setting and observed stronger deactivation in the DMN in the nonreactive group. This evidence is not in line with simply reduced engagement during the n-back task, although we cannot rule

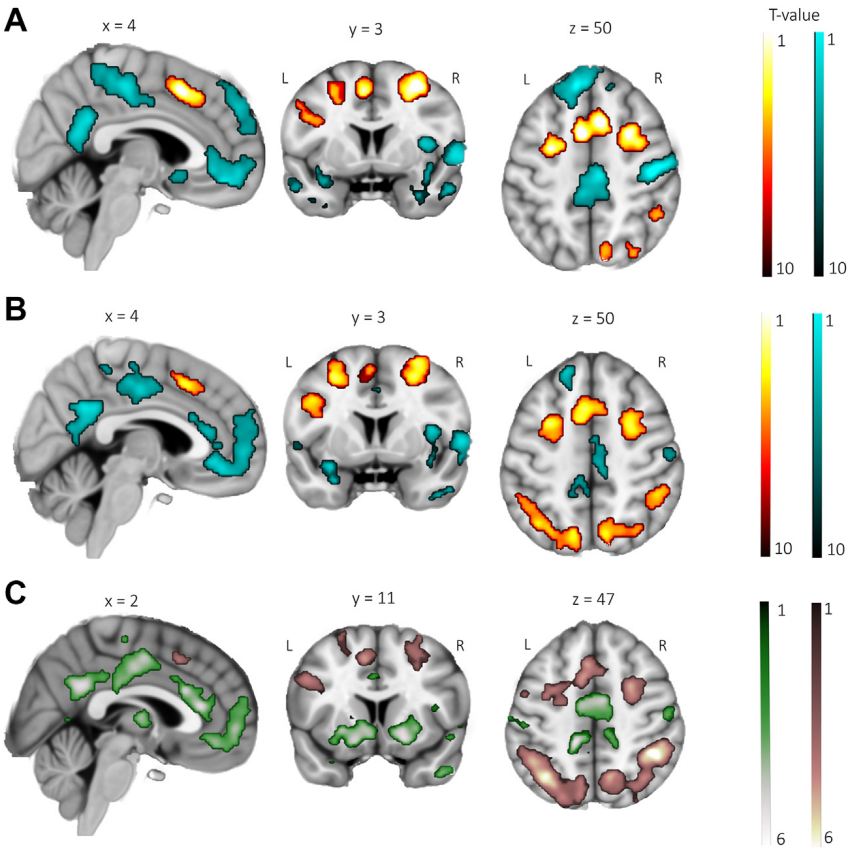

Figure 2. **(A)** Neural correlates of mean pupil size for the reactive pupil response profile (n = 47) between conditions. Red indicates blood oxygen level–dependent (BOLD) activity positively correlated with pupil size. Cyan indicates BOLD activity negatively correlated with pupil size. $p_{voxel.FWE} < .05$, k > 50. **(B)** Neural correlates of mean pupil size for the nonreactive pupil response profile (n = 47) between conditions. Red indicates BOLD activity positively correlated with pupil size. Cyan indicates BOLD activity negatively correlated with pupil size. $p_{voxel.FWE} < .05$, k > 50. **(C)** Interaction contrast between the reactive and nonreactive group. Green indicates reactive > nonreactive. Pink indicates reactive < nonreactive. $p_{cluster.FWE} < .001$, k > 100 (voxel collection threshold: uncorrected $p < .001$). x, y, z in Montreal Neurological Institute space. FWE, family-wise error; L, left; R, right.

out this explanation completely given the indirectness of the evidence. With this study, we extend the existing literature on depression-related pupil reactivity alterations, where individuals with depression or heightened risk for depression display stronger arousal toward negatively valanced stimuli (32). The pupil reactivity profiles are in line with hypoactivity and hyperactivity patterns in PFC and limbic system regions of the brain in depressed individuals (56). Another study using computational neural network modeling on the Stroop task showed decreased pupil dilation, which was consistent with disruption in PFC functioning leading to decreased cognitive control and vigilance toward the task at hand (57).

Further, we were able to biologically validate the pupil response profiles with fMRI. First, we mapped the neural correlates of mean pupil size across cognitive load conditions separately in each of the two pupil profile clusters and demonstrated positive correlations in the FPN and negative correlations mainly in the DMN, next to the caudate, amygdala, and hippocampal regions, replicating and extending our previous study in healthy control participants (25). This shows that in general, the increase in pupil size is temporally coupled to WM-related brain processes in both groups. Interestingly, the differential contrast revealed that the attenuated pupil size patterns in the nonreactive group were characterized by stronger activation in FPN regions and stronger deactivation in the DMN, despite decreased behavioral performance. The question arises: Why did we observe this at first glance contradictory result? One answer is that, potentially, the reactive group manages to successfully upregulate the arousal system, which becomes visible in both the pupil reaction and its coupling to activity in the thalamus. In contrast, the nonreactive group is failing to do so, and as a consequence we observe a compensatory pattern with increased, but inefficient recruitment of the FPN and stronger deactivation of the DMN, resulting in poorer behavioral performance reflected in reaction times of the n-back task. A recent meta-analysis indicated that any cognitively demanding task activating the executive control network, namely the FPN, downregulates the amygdala in a load-dependent fashion, postulating a reciprocal relationship (58). Pupil dilation is coupled to these brain regions, as the task tracks not only mental effort, but also arousal-related processes (16). The deactivation of the DMN (when considered in relation to the pupil reaction) might also be an indicator of the WM load interpreted as too high after an inefficient arousal reaction and represent a consequence of the lack of arousal. In sum, the nonreactive group might not recruit optimal levels of arousal and mental effort, which is evident in their brain activation and pupil responses.

The role of the LC-NE system and the results on the effect of mental health status on the individual pupil response profiles in relation to neurocognitive functioning are discussed in the Supplemental Discussion.

Although we have robustly validated our analysis and also showed biological and behavioral correlates, our LCGM result should be validated in an external sample, ideally with the same experimental setup. Moreover, further validation using the IPP, mapping the pupil size increase between a baseline condition and more demanding condition, in other samples would also be a valuable endeavor. As we derived for each participant IPP scores based on the conditions from the widely used n-back task, which are independent of clustering procedures or sample compositions, we aimed to increase the external validity of this study and facilitate replication. Further, it could be of special interest to run these analyses in samples with stronger impairments on the cognitive and affective and anxiety symptom level, e.g., individuals with severe depression in inpatient care. In our case, we could not show any significant associations with symptom severity related to depression and negative affectivity, which could be due to the fact that we included unmedicated, high-functioning participants with varying degrees of affective and anxiety symptomatology. In turn, this is also a strength of the current study, as we were able to observe psychophysiological patterns without the possible confounding effects of pharmacological treatments. The fact that in our sample we did not observe severe impairments in participants could be the reason for the different size of the clusters, as fewer participants automatically belong to the nonreactive group. An additional strength of our study is that we examined participants across diagnostic criteria within the affective and anxiety disorder spectrum, moving beyond single disorders to a broader scale analogous to the Research Domain Criteria (59). Despite the fact that we were able to successfully induce varying WM load conditions as reflected in the behavioral readouts (see 2.8 in the Supplement), future experimental designs could benefit from implementing conditions with higher load to potentially observe an asymptotic reactivity pattern in pupil fluctuations.

In conclusion, we found two robust clusters with different pupil response profiles that were related to neurocognitive functioning in the domains of executive control and sustained attention. Through the simultaneous measurement of pupillometry and fMRI, we were able to dissect an arousal pattern during cognitive processing and highlight its necessity of upregulation and downregulation for successful performance. Pupil dilation has the potential to act as a physiological readout for neurocognitive performance in the domain of executive functioning, a core construct in psychopathology (1). The data-driven investigation of potential physiological markers of neurocognitive functioning in mental disorders could inform clinical research, and further facilitate diagnostic models and personalized treatment decisions.

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5

Conclusion

Psychological states, such as mood, alertness, and motivation covary with activity in the neuromodulatory systems of the brain impacting behavior. As such, they have a substantiated effect on neurocognitive processes of sustained attention, perception, and the ability to both retrieve memories and create new ones. Many pscychiatric and neurodegenerative disorders are related to dysfunction in these neuromodulatory systems. The LC constitutes one of the major sources for NA, a neuromodulator which plays a central role in higher order neurocognitive processes involving the PFC and OFC (Strauch et al., 2022). Elucidating the factors that influence the LC and its subsequent pathways and mechanisms is valuable for the understanding of how the brain allocates attention and adapts to the environment to select, store and retrieve information in order to form adaptive behavior (Sara, 2009).

The studies presented as part of this thesis recorded pupil diameter and BOLD activity simultaneously, which allowed capturing moment-to-moment changes in neurocognitive processing and attentional effort and enabling a precise modelling of the relationship between pupillary responses and brain activity. The first project contributed to the understanding of underlying cognitive load mechanisms in revealing two specific subprocesses, one related to working memory and the other one to task-related salience (Fietz et al., 2022).

The second project proposes the use of pupil size recordings as a valuable tool for the assessment of neurocognitive functioning in a transdiagnostic sample encompassing healthy individuals as well as patients with mainly affective and anxiety disorders. Pupil response profiles, identified through data-driven clustering, were strongly associated with the performance in neurocognitive measures tapping into the domain of executive functioning (Fietz et al., 2023).

Therefore, the pupil has the potential to support diagnostics of neurocognitive deficits, in particular in an early stage of the decline. As pupillary constriction and dilation provide an easily accessible, inexpensive, and noninvasive readout they offer a diverse range of applications, specifically in the clinical field (Strauch et al., 2022). The early detection of alterations in pupil size could indicate symptoms such as poor concentration, high arousal levels, and working

memory impairment which are common across various psychiatric and neurodegenerative disorders (Abramovitch et al., 2021). Future research could follow-up on the results and implement and expend the experimental setup to other psychiatric and neurodegenerative disorders as the LC-NA system is implicated in various conditions. For example, in attention-deficit hyperactivity disorder executive functioning is impaired due to neuromodulatory influences over fronto-cerebellar circuits while showing disorder specific pupil fluctuations (Del Campo et al., 2011; Nobukawa et al., 2021; Townes et al., 2023). These pupillometric signatures could be used for the assessment of neurocognitive processing in these conditions as well as potentially give insights on changes in various brain states influenced by, for example, pharmacological interventions.

To provide a more nuanced view on the presented studies in this thesis and its contribution to the field it is important to address the limitations. Generally, cross-sectional study designs, such as the BeCOME study, are comparable to a snapshot where acute and chronic cases, which are known to exhibit substantial differences, are pooled together. The varying disease severities and etiologies may shape the results in different ways and in principal do not allow any deductions which tap into causal interpretations (Spector, 2019). Additionally, from an experimental psychological view, it could be of interest to add conditions with a higher cognitive load to the experimental setup. This could allow potentially the observation of the inverted U shape in working memory performance as well as the related findings in NAergic neurons and its manifestation in pupil fluctuations (Aston-Jones & Cohen, 2005).

The overarching goal of neuroscientific research in the field of psychiatry and clinical psychology is the establishment of biomarkers to inform and individualize diagnostics and treatment for patients. Until now, neuropathological underpinnings of psychiatric disorders have little to no influence on current healthcare practice as clinical decisions are mainly based on phenomenological profiles (Cuthbert & Insel, 2013). The question is what characteristics a potential biomarker would need to be considered in healthcare practice? Should it completely substitute the phenomenological approach or rather add on valuable information? In other medical fields, in which biomarkers are already well established, they provide an objective estimate of the disease. The target conceptualization of a biomarker would be that it needs to be reasonably simple and fast to obtain so that it can be easily implemented by clinicians. Additionally, the personal and economic costs should be low and would ideally contribute to the decision on which treatment is most likely to be beneficial for the patient (Jollans & Whelan, 2018). For decades no such biomarker with sufficient evidence was identified in psychiatry that allows the questioning of biological reductionism within “brain disorders” in general. One idea presented in the past years was to reorganize the phenomenological conceptualization of psychiatric disorders within a network model. The hypothesis is that psychiatric disorders are rather a causal interplay of symptoms which are highly context depended with multifactorial explanations (Borsboom et al., 2019). However, setting aside the biological substrates of medical conditions might potentially further hinder the progression of personalized medicine within psychiatry. A paradigm which incorporates biological mechanisms, and their behavioral manifestations is the process-view of psychopathology. Within this notion, psychiatric disorders are rather disconnected from the current problematic artificial labelling and manifest within processes such as reward anticipation, salience monitoring, fear conditioning and executive

functioning. These processes are functional entities on a continuum where their nature matches with the dimensionality between health and disease (Elbau et al., 2019; Kopf-Beck & Fietz, 2021).

The work summarized in this thesis introduces such a process-oriented physiological marker using pupillometric data reflecting executive functioning abilities across psychiatric disorders, therefore, having the potential for identifying related malfunctions before its manifestation in behavioral responses. Changes in coupling between NA release and pupil fluctuations could reveal impactful insights into the effects of stress on brain states, specifically related to the involvement of the PFC. Differing levels of NA provide a molecular switch for whether the PFC and consequently the amygdala are engaged or weakened. For example, patients with post-traumatic stress disorder show elevated NA levels in cerebral spinal fluid concentration (Geracioti et al., 2001). The PFC regulates levels of arousal though projections to NA neurons where it can inhibit or facilitate LC firing and reduce or amplify the stress response (Arnsten et al., 2015).

This process-view moves away from dichotomous categorization of health and disease and takes social and normative factors into account, tapping into a rather holistic view of the human disposition. A multimodal approach spanning wide-ranging levels of assessment for studying pathophysiology could enhance the identification of individual biologically derived adaptive and maladaptive patterns (Brückl et al., 2020). A combination of psychophysiological, functional, and anatomical studies are needed to gain a better understanding of these process-oriented mechanisms which form human behavior, mental, and emotional states (Elbau et al., 2019). The main neuromodulatory systems in the brain play a crucial role in essential physiological, behavioral, and neurocognitive functions, and have been linked to various psychiatric disorders. Although, precise techniques for investigating neuromodulation, specifically NA recordings, exist in animals, similar advancements are required in humans to expedite translational research and further enhance the comprehension of the continuum between health and disease (Bang et al., 2023).

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